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No. 3 CANCER OF THE CERVIX
Diagnosis of Early Forms

CIBA FOUNDATION
STUDY GROUP No. 4

Virus Virulence and Pathogenicity

in honour of
Prof J MULDER

Editors for the Ciba Foundation

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The purpose of the Ciba Foundation, which is to promote international co operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry Its domicile in London is indicated by the sword of St Paul and the British lion, the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town

PREFACE

The Ciba Foundation, a unique international institution, owes its inception to the generosity of CIBA Limited, Basle However, being established under British trust law, it enjoys complete independence in practice and policy

Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place Foremost in its activities is the organization of small conferences, some lasting 3–4 days, others for one day only The proceedings of the former are published in book form, and the latter in the manner of the present booklet Each of these one-day study groups is being organized in honour and around the work of an eminent member of the Foundation's scientific advisory panel, on which there are 1–3 representatives for most of the countries of the world

The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture An exchange programme between French and British postgraduates is conducted and a library service is available Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research

The publication of the first four study groups is regarded as experimental Nevertheless with rapid production of the proceedings of both conferences and study groups, it is hoped that readers in more distant centres, away from easy reference to the latest literature, can feel a little more closely in touch with recent developments and may be stimulated to make their own topical contributions to international research

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CHAIRMAN'S OPENING REMARKS

SIR MACFARLANE BURNET

IT is an honour and a pleasure to be asked to chair a discussion in honour of Prof Mulder, particularly on a subject which he has made especially his own

The central interest of our discussion is the pathogenicity and virulence of influenza virus, with other viruses included, more or less to throw light on that theme of Prof Mulder's Among the themes in microbiology which have human implications the virulence of viruses is probably outstanding The influenza pandemic of 1918 was, apart from the classical bubonic plagues the greatest pestilence that has ever afflicted the human race and most of us were impressed recently by the picture of the age incidence of the 1957 Asian influenza in America It seems that we may have just barely escaped another 1918, because there was a

Everyone here will agree that we are up against a major difficulty in talking about virulence It is not an absolute character It is wholly dependent on the relationship between host and parasite, and both will be considered by speakers here

My own special interest for the last ten years has been the genetic aspects of influenza virus There is no doubt that we can detect differences in virulence amongst strains acting on the same host, and that these differences in virulence are inheritable and hence genetic characters During the last ten years I have attempted to define in genetic terms what is meant by virulence in influenza virus at the experimental level In fact this was the subject of a Ciba Foundation Lecture which I gave a few years ago (1954, *Lancet*, 2, 559) But there is no simple answer There are no virulence genes as such, and my present feeling is that virulence must be considered as a sort of integration of all the characteristics

of the virus which are related to its capacity to infect and multiply in the cell, and therefore must always be expressed in terms of the [illegible]

in mind that the genetics and the population dynamics of any pathogenic organism are determined essentially by the way in which the virus survives as a species and not by its human pathogenicity Virulence is, as it were, an epiphenomenon of the processes by which the virus survives in Nature in relation to the full totality of the environment, and where difficulties begin to arise in discussion of the present kind, we can often clarify them by using this ecological approach

THE DEFINITION AND MEASUREMENT OF VIRUS VIRULENCE

C. H. Stuart-Harris

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Virus virulence is almost indefinable and practically impossible [illegible]

The fact that we have no better word than virulence to describe the means of production of disease by viruses should surely also mean that it is not a useless symbol or abstract concept. The attribute of being poisonous which is the factual meaning of virulence becomes all the more real when substances, organisms or viruses are found which do not possess this property The fact that some viruses are non-virulent is probably the chief reason for attributing variations [illegible] papers in this study group In truth virulence is an attribute of a micro-organism which can only be measured and defined by reference to a host system

The definition of virulence

It is first necessary to consider whether the word virulence should or should not be regarded as synonymous with pathogenicity. Most workers do in fact use the words in an alternative sense, but Miles pointed out in 1955 that it was useful to regard pathogenicity as an attribute of a species, genus or some other grouping of parasites and to reserve virulence for the expression of the pathogenicity of a given stable line of micro-organism. There are good grounds for agreeing to this distinction in the case of viruses particularly because of the very wide variations in the behaviour of different strains of many animal viruses. With this background it is possible to define pathogenicity as the power to

produce pathological effects in a host, and virulence as the evidence of pathogenicity derived from observation of the symptoms and signs, degree of illness or death of the host.

The measurement of virus virulence

(a) The incidence of disease

Attempts have been made to measure virulence with varying degrees of accuracy by studies of whole populations, of individual patients or by experimental study in the laboratory upon animals. Let us first examine the facts obtained from a study of human illness in whole populations. There are certain characters which can be measured in a population, such as the incidence and mortality from an infectious disease, and these are often used as indices of the degree of virulence of the infection. It is, however, obvious that these indices cannot be divorced from the effects of age, for age introduces a variability into the equation and prevents a direct comparison between two populations unless these are identical in structure.

that immunity following childhood infection persists either by means of antibodies or by some change in the cellular resistance to attack. When a population which has been previously unexposed to an infectious illness becomes infected the age incidence of disease is said to be quite different from that of the acute specific fevers, children and the older adults may suffer less severely than young adults (Burnet, 1953). Thus, the age incidence does not give a clear picture of host resistance unaffected by the specific effect of previous contact with the infectious agent. When, therefore, a disease changes its age incidence it may be difficult to say whether this change is due to a variation in the host or in the parasite.

Poliomyelitis may be chosen as the example. Here is a striking example of altered attack rate and of age incidence in our own lifetime. For many this is only reasonably explained by invoking an alteration in virulence of the poliovirus. Others, however, point to serological differences between populations which have no epidemics of poliomyelitis and those in which the disease is epidemic. The later development of specific antibodies in the

children of the populations experiencing epidemics points to a lack of exposure to the virus in early infancy which would bring about herd immunity There is then no need to invoke an alteration in the virulence of the virus to explain the evolution of poliomyelitis at the present time

Poliomyelitis, however, illustrates another aspect of age which is of great significance This is that with increase in age there is increasing severity of illness and of mortality This is seen clearly by reference to statistics from this country or from a population such as the Eskimos who have infrequent epidemics (Peart, 1949) Sabin (1956) explains this by suggesting that the motor neurone of the adult is more susceptible to attack than that of the child

In typhus fever, too, mortality is not related to susceptibility to infection but is clearly correlated with age In the Naples epidemic in 1943–44 the largest number of cases was in children and young adults in whom the mortality was about 5 per cent (Table I) In those over 30 years of age mortality rose sharply even though the actual number of cases was less (van den Ende *et al*, 1946) Even in the Algerian Arabs among whom previous exposure to endemic typhus might well have modified severity, the same trend of mortality with age occurred as in their European neighbours (Table II) One can perhaps explain this increased mortality from typhus in older persons as being due to the failure of the ageing circulatory system to resist the attack made upon it by the rickettsia

So far as the respiratory tract is concerned it is a familiar fact that young infants and the aged are subject to the highest mortality and young adults the least Even in an area where infection has been absent for many years and is then reintroduced the age distribution of mortality may follow the same pattern This is well shown by the measles epidemic of the Faröe Islands in 1846 which occurred sixty five years after the previous epidemic (Table III) The incidence was the same for all ages, being 77 per cent of all those at risk who did not have the disease in 1781 But the mortality was greatest (28 6 per cent) in infants under 1, next highest in adults over 80 (16 3 per cent) and negligible in those from 1 to 30 years of age (Panum, 1940) Of course, this mortality must represent the combined effects of virus and nasopharyngeal bacteria on the respiratory tract

Table I

The Naples Epidemic—sex and age and mortality December 1943–February 1944

Age groups	*Males*	*Deaths*	*Females*	*Deaths*	*Total numbers*	*Deaths*	*Mortality per cent*
Under 3	20	1	18	0	38	1	2 6
3–11	133	2	91	1	224	3	1 3
12–20	221	9	166	10	387	19	4 9
21–29	105	11	108	10	213	21	9 8
30–38	111	14	127	18	238	32	13 4
39–47	59	29	116	28	175	57	32 5
48–56	43	18	56	19	99	37	36 3
57–65	10	7	27	15	37	22	59 4
66–74	2	2	8	3	10	5	50 0
75 and over	1	1	1	1	2	2	100 0
Gross	705	94	718	105	1,423	199	13 9

Table II

COMPARISON BETWEEN TYPHUS IN THE LONDON FEVER HOSPITAL 1848–1870 ALGIERS 1942 AND NAPLES 1943 1944

	London 1848 1870		Algiers 1942 French		Algiers 1942 Arabs		Naples 1943 1944		
Age groups	*Numbers*	*Mortal ty per cent*	*Numbers*	*Mortal ty per cent*	*Numbers*	*Mortal ty per cent*	*Age groups*	*Numbers*	*Mortal ty per cent*
0–9	1 430	4	20	10 0	76	6 6	0–11	262	1 5
10–19	5 121	3 5	72	7 0	317	7 3	12 20	387	4 9
20–29	4 127	12 3	62	17 8	620	9 2	21 29	213	9 8
30–39	2 976	23 2	79	32 9	368	18 4	30–38	238	13 4
40–49	2 546	35 5	79	44 5	246	26 0	39–47	175	32 5
50–59	1 231	51 1	73	67 2	111	50 5	48–56	99	36 3
60–69	588	65 1	} 42	} 81 0	} 54	} 53 6	57–65	37	59 4
70–79	116	75 8					66 and over	12	58 3
80 and over	3	100 0							
Gross	18 268	18 92	457	35 8	1 846	16 8	—	1 423	13 9

Table III

FARÖE ISLANDS MEASLES EPIDEMIC 1846

Age groups	*Population*	*Number attacked*	*Deaths*	*Case fatality per cent*
Under 1 year	198	154	44	28 6
1 9	1 440	1 117	3	0 3
10–19	1 525	1 183	2	0 2
20–29	1 470	1 140	4	0 3
30–39	842	653	10	1 5
40–49	791	613	19	3 1
50–59	728	565	27	4 8
60–69	480	372	27	7 1
70–79	272	211	19	9 0
80 and over	118	92	15	16 3
TOTAL	7,864	6 100	170	2 8

Influenza is a disease whose age variations in different epidemics are regarded as classical examples of the effect of changes in the virulence of the causative virus Clinicians faced by the awe inspiring spectacle of the winter pandemics of 1918–19 were familiar with the former innocence of influenza in young adults. They were therefore bound to explain the excessive mortality in young adults by postulating a change in the virulence of the infecting virus Yet Francis (1953) has sought to interpret the changed mortality in 1918 as an expression merely of the intensity of infection In his view the sparing of older persons from a high attack rate was due to previous experience of the virus antigen which young adults lacked Francis suggested that the case mortality from influenzal pneumonia increased steadily with age in 1918 just as it d d in epidemics before or after the pandemic Even if we were able to accept that the 1918 pandemic caused an exceptionally high incidence of infection in young adults the recent experience of the Asian pandemic of 1957 should surely make us hesitate to follow Francis' opinion for in 1957 the high attack rate in children and young adults experienced all over the world was not accompanied by any comparable incidence of pulmonary complications or mortality Thus the remarkable prevalence of pneumonia in young adults in 1918 is a phenomenon which cannot readily be explained except by reference to the biological properties of the pandemic virus No doubt members of this study group will wish to refer again to this epidemic experience and will give their own explanations

(b) Observation of individual illnesses

As it seems to be difficult to arrive at an estimate of virulence from the study of whole populations, let us next examine the evidence derived from study at a clinical level on individual patients This is the technique used by those who seek to estimate pathogenicity by the use of human volunteers The latter are never so numerous as to constitute a population, and consist merely of a handful of individuals Something indeed can be learned by this method, as we shall hear later in this study group, but the technique is extremely limited in application

Meanwhile, clinical studies have shown the importance of host factors other than specific or immunological resistance which determine the outcome of infection Nutritional status is widely believed to be such a determinant particularly in the case of bacterial infections such as tuberculosis (Dubos, 1955) and Salmonella infections (Schneider and Zinder, 1956) Yet the findings in the case of virus diseases seem to be contrary to those of bacterial infections If it is true, as it seems to be that the bonniest children often fall victims to poliomyelitis, is this an argument that those who are undernourished are thereby protected from paralysis? Social circumstances are of such great importance in determining the spread of poliovirus and thus of aiding subclinical immunization that one must beware of drawing hasty conclusions But the suggestion has certainly been made—and there is no easy way either of refuting or of confirming it from human experience—that some virus infections are more trivial in the under than in the over-nourished host

Until quite recently the importance of the endocrines on the outcome of infections was minimized Now, however, cortisone has come along and it is possible to observe the effects of steroid administration on man as well as on animals with experimental infections Does the enhancement by cortisone of poliomyelitis in the hamster (Shwartzman 1950) or of Coxsackie virus infection in mice (Kilbourne and Horsfall, 1951) have human analogies? One can point of course to the frequent severity of poliomyelitis in pregnancy, though this is not entirely undisputed Perhaps Haggerty and Eley's observations (1956) on the excessive severity of varicella in children treated with steroids is analogous to the results with viruses in cortisone treated hamsters and mice Yet these are rare human happenin and one would indeed be bold

suggest that endocrine changes are of much concern in influencing the outcome of the common virus infections

Perhaps it is even more important to stress that host resistance is far more complex than this reference to nutrition and to metabolism would suggest The clinician now has it in his power to ameliorate or to prevent infection as when he uses gamma globulin to attenuate or to abort measles Unfortunately virus infections appear to involve a form of resistance which is not humoral or else children with agammaglobulinaemia would perish from the common cold or influenza. The studies of Good and Zak (1956) and of MacCallum (1959) on agammaglobulinaemia show quite clearly that most virus infections and even vaccination take a normal course in children stripped of the barrier of specific immunological protection.

So, like the studies in populations, clinical studies afford only a glimpse of the relative virulence of viruses and one turns with hope to the experimental study of virulence in the laboratory.

(c) The experimental measurement of virulence in the laboratory

The first matter to be decided by the virologist is the host species which will be employed. This is because the adaptation of a virus to a new host is linked with the acquisition of virulence for the new species and perhaps with a loss or attenuation of virulence for the former host species Yet mere choice of species is not enough Miles (1955) when referring to the measurement of virulence in micro-organisms stated that one can only determine the comparative effect of two organisms or two strains of the organism in the same set of hosts He inferred that there can be no such things as a quantitative expression without a standard of reference Furthermore, the comparison of virulence is only possible if one can measure the dose or quantity of virus or other organism present in the preparations inoculated into the hosts This matter of quantitation is, of course, the great stumbling-block for virologists No matter whether we use 50 per cent infectious doses, or haemagglutinating units, or some other measure, it has to be admitted that it is far less precise than one would wish. Moreover, with attenuated strains of virus one is dependent upon means of estimating infectious units in some system other than the host under study

The next requisite, when the host and the unit of measurement of the virus have been decided upon, is to define the set of conditions under which variation of the response of the host will be minimized It may be necessary to define the genetic constitution of the host in addition to the species This was clearly shown by Sabin (1952) when he discovered that the Rockefeller Institute breed of mice are insensitive to the 17D strain of yellow fever virus regardless of dosage Swiss mice are, by contrast, uniformly susceptible to this strain of virus Sabin found that the resistance of the PRI mice also extended to other neurotropic viruses and explained the lack of virulence of 17D virus as being due to the low level of multiplication achieved by this virus in the mouse brain He further showed that suckling mice of the PRI strain were susceptible to 17D virus up to four days of life, and age is certainly a factor of the greatest importance in host resistance in the laboratory as in man

One thinks, of course, of the Coxsackie viruses as being the best example of limitation of pathogenicity by the age of the host, but an enhanced susceptibility of young animals is found with many viruses (Sigel 1952) Clearly, age does not matter when virulence

The route of inoculation is the third factor which requires standardization Route does not necessarily matter if one is studying viruses capable of a wide dissemination throughout the body but it is vital in many other instances because of particular tissue tropisms Influenza virus affords excellent illustrations of the importance of the route of inoculation in studying the virulence of different strains The need to bring the virus into direct contact with the cells of the respiratory tract or of the nervous system in the case of neurotropic strains is only obviated when using baby mice The fact that subcutaneous influenza virus does not initiate infection in adult mice prevented me for some time from recognizin that I was dealing with an instance of infection by the neurotropi WS virus in suckling mice I then found that the virus coul produce encephalitis with nervous system lesions and death up t

about the twelfth day of life Tyrrell and Cameron (1957) later showed, however, that lung adapted strains of influenza virus such as WS and MEL could also kill suckling mice after subcutaneous inoculation though lung and not brain lesions were then produced Clearly some form of barrier develops with maturation of the host

ferent ages (Sabin and Olitsky, 1937)

Finally, the effects of the viruses on the host must be measured in a standard manner Mortality is obviously preferable to lesions unless the latter are sufficiently characteristic and can be graded quantitatively Unfortunately, lesions frequently give only crude indices of effect and estimates of the degree of virulence of attenuated *viruses are correspondingly approximate*

Practical problems concerned with the measurement of the virulence of the poliovirus

The importance of all these matters concerned with the attempted measurement of virus virulence can best be appreciated by reference to practical problems such as the use of virus vaccines for human immunization This will be illustrated here from the standpoint of poliomyelitis, which has in the recent past caused virologists to think a great deal about the definition and measurement of virus virulence

The drive to produce a living attenuated poliovirus vaccine which can be used safely in man requires an estimation of virulence in two connexions First, it is necessary to find strains of poliovirus which cause no harm when fed to man via the alimentary route Secondly, it remains to be shown that the living viruses excreted by those to whom the vaccine strain is administered, are not hazardous to those in contact with the vaccinated subjects Both needs have been explored by means of comparison of the properties of the viruses in monkeys and chimpanzees inoculated by various routes Both of these practical problems have led to controversy

Of the three variables mentioned already in the preliminary discussion, the choice of host has not been in doubt for most workers Rhesus or cynomolgus monkeys are the only practicable test species for determining the virulence of the viruses for the central nervous system Mice are only useful for Type II strains and chimpanzees

are too precious This means that most work on the measurement of the virulence of poliovirus has been simply a study of virulence for the monkey often on only small numbers of animals Now Sabin (1956) has pointed out that the virulence of polioviruses for the monkey is not an all-or-none character but that viruses derived from healthy children or after various laboratory manipulations can [illegible]

most virulent strains can reach the central nervous system after peripheral inoculation, others only produce an effect when large doses are inoculated directly into the brain, and the most attenuated viruses produce no paralytic effects after intracerebral injection but may in very large doses still paralyse the motor neurones of the spinal cord after intraspinal injection into the lumbar enlargement According to Sabin even strains capable of causing some paralysis after intracerebral injection into monkeys are without effect after intraspinal injection into chimpanzees

In his recent publication, Sabin (1959) shows the relative effects of various Type I polioviruses after intraspinal inoculation into cynomolgus or rhesus monkeys If it is thus possible to measure virulence with such apparent precision, it should be equally possible for different observers to obtain good agreement between their results Unfortunately this is not true and Sabin considers that the discrepancy between different observers is explained by the fact that reproducible results demand scrupulous attention to technical detail particularly with intraspinal inoculations

A second discrepancy confronts us, however, in regard to reported properties of the viruses excreted in the stools of volunteers fed attenuated viruses by mouth. In Belfast, the attenuated Types I and II viruses of Koprowski gave rise to excreted viruses with power to paralyse monkeys after intracerebral inoculation (Dane *et al*, 1957, Dick *et al*, 1957) In Sheffield, Sabin's Type III attenuated virus was fed to children, some of whom became infected and excreted virus in the stools Comparison of the virulence of the excreted virus with the original vaccine strain led Clarke and her co-authors (1958) to conclude that the viruses had attained enhanced neurotropism for the monkey during passage through the alimentary canal Sabin himself (1957) has found the

virulence of excreted viruses to be *perceptibly different from that* of the vaccine strains but considers that the quantitative change is not such that the viruses constitute a hazard for man He bases this deduction on the results of intraspinal inoculation into chimpanzees which suggest that the strains with residual paralytic properties in monkeys are harmless when given intraspinally to chimpanzees Nevertheless the fact that there is some sort of

arly under the circumstances of infection of the human alimentary tract This over a period of weeks resembles a series of passages in a culture in the laboratory and thus affords the maximal oppor tunity either for genetic variation or for selection of strains with particular properties

because of this efforts have been made to estimate virulence in a host system capable of more rigid control such as is possible in tissue cultures This brings us to a discussion of virulence at a cellular level

Virulence at a cellular level

The present phase of virus work has given impetus to the study

systems to provide markers which can be equated or even sub stituted for tests in animals is refreshing It accounts for the dis covery that attenuated polioviruses may multiply to a different extent from virulent viruses in cell cultures held at a certain pH (Sabin 1956) Vogt Dulbecco and Wenner (1957) have indeed suggested that growth of virus in cells at different *acidities may be* a character which is genetically linked with that of the virulence of poliovirus for the monkey Kanda and Melnick (1959) have also

shown that virulent and avirulent poliovirus strains may grow to differing titres in different cell lines. Yet it cannot be said that tissue culture systems can be substituted for whole animal hosts in the measurement of virulence, for there is a wide gulf between the effects observed *in vitro* and *in vivo* with the same virus

A given strain of influenza virus may have a high order of virulence for the mouse lung in living mice but may not attack the cells from a mouse lung grown in tissue culture Again environmental factors of almost trivial importance appear to affect the destructive action of viruses on cells Thus, Frothingham (1959) has recently shown that growth of the egg-adapted Type II, MEF strain of poliovirus in human amnion cells is much affected by the age of the culture and by motion of the tubes The virus is cytopathic in old, but not in young, cells and in rolled tubes but not in stationary cultures

The profound effects of alteration in the biochemical environment of tissue cultures on the resultant growth of viruses is known through the work of Prof H R Morgan He has shown how one can convert cells in tissue culture into resistant hosts for psittacosis virus by starving them of essential nutrients (Johnson and Morgan, 1956) It would be necessary to control all conceivable factors affecting cell growth *in vitro* if a stable reproducible host system were to be elaborated Perhaps it was knowledge of the modifying effects of environment on the host virus relationship which led the virologists attending the Wisconsin symposium on latent virus infections in 1957 to refer to the destructive effects of viruses on cells in culture as "cytocidal". They reserved "virulence" for observed pathological effects in whole animal hosts Ought we to do the same?

DISCUSSION

Tyrrell I would add the suggestion that in a laboratory study one should always use, if possible a single genetic clone or group of virus particles, because it is probable that one of the vitiating factors in this work is that we are dealing with a mixture of different kinds of viruses We do one test and measure the properties of one component, the next time we do another test and measure the properties of another component of the mixture

Burnet I should like to underline that suggestion very strongly

Morgan This is also true when you have the advantage of doing *in vitro* experiments in [illegible] ones of

you start off with a differentiated kidney cell, by the end of a few days it is rather different, and, as Frothingham (1959) showed, it goes on

Tyrrell No

Kilbourne Lest we deprecate the intact host too much, we must remember its "built in" homeostasis which we do not have in tissue culture

Goffe There is also the disadvantage that the virus as excreted by the intact host is not a single clone If you want to examine the virus

polate from tissue cultures to the whole hosts In 1957 Dr Chapron
iere and I studied the susceptibility to myxoma virus of guinea pig human and other tissues in culture We found that cells of all kinds of species were susceptible At that time we found in the literature only one or two other examples of a non susceptible species whose cells would grow a virus in tissue culture Since then it has become

virus

Smith One should also be cautious about accepting these so called clones as being relatively uniform How quickly do they change? From work we have done it seems that in spite of the use of what is

population I am quite sure that is not so

heterozygous I am confident that many influenza virus particles are heterozygous i e carrying two or more alternative genomes It has yet to be proved whether or not the poliovirus is heterozygous That introduces a major difficulty into the question of producing and maintaining pure clone virus

brought the disease home In homes where old people and young children had a chance of mixing, the old people got influenza just as

undefined

Dick Is the actual quantity of virus multiplication known? We know that, in poliomyelitus, the virus multiplies better in small children than in adults given the same dose of virus Does that

HOST CELL FACTORS AND VIRUS VIRULENCE

HERBERT R MORGAN

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IN the interaction of a virus with its host cell, both members of this complex have an important rôle in determining the outcome which is a measure of the virulence of the virus, and each in turn is subject to factors in the microenvironment in which this contact occurs In considering the rôle of host cell factors, it is desirable to minimize the role of environmental factors such as antibody and therefore a restriction of this discussion primarily to *in vitro* systems has many advantages The *in vitro* system permits the study of a given virus with a defined cell system in which all of the cells are subject to direct observation and the extracellular environment is subject to control Also the *in vitro* system makes it possible to simplify the definition of virulence which will be taken to mean any visible effect produced on the host cell which will include not only cell destruction (cytopathogenic effect, CPE) but cellular alterations associated with continued cell replication as produced by certain tumour viruses Our growing knowledge of these latter agents requires that they receive recognition as an important group of viruses that possess certain unusual characteristics, the most striking of which is their capacity to stimulate the unrestricted multiplication of the host cell *in vivo* Admittedly their effects on cells *in vitro* are not compatible with the more classical concept of cytopathogenicity based on cellular destruction, though under certain circumstances they may exhibit this action in tissue culture

The interaction of the virus with its host cell may have several general forms (*a*) the virus may multiply and produce a cyto pathogenic effect (CPE), or (*b*) it may produce a CPE without the production of infectious virus though there may be evidence of the synthesis of viral components by the host cell In both of these

cases, the virus would be considered virulent *in vitro*. On the other hand, the virus may (*c*) multiply without observable effects on the cell, or (*d*) fail to multiply and to produce an effect on the cell and be considered avirulent in either case.

The failure of completion of the infectious cycle by a virus in contact with a cell may occur at a number of different phases in this cycle, i.e., adsorption, penetration, eclipse, viral replication and release. Cellular factors may influence most of these events in a decisive manner.

Host cell factors in viral adsorption, penetration and eclipse

The susceptibility of freshly isolated cells to virus infection and subsequent destruction has been extensively studied with poliovirus infection of monkey kidney cells. Kaplan and Melnick (1955) showed that kidney cells from the South American capuchin monkey supported the growth of Type 1 poliovirus without evidence of a CPE and thus the virus might have been thought to be avirulent for these cells. However, it was shown that the cell population adsorbed only a small part of the virus inoculum (Kaplan, 1955) and that only about 1 cell in 2000 was susceptible to virus infection. These few infected cells accounted for the viral replication. Obviously the lack of a CPE was due to the fact that the destruction of a small number of cells in the large population could not be detected. Failure to adsorb virus rendered it avirulent for the majority of cells. However, the adsorptive capacity of the cell for poliovirus is not the only factor important in the susceptibility of kidney cells from different monkey species, since patas and rhesus monkey renal cells adsorb virus equally well, but a larger number of plaques appear on the patas cell monolayers. This indicates a greater degree of susceptibility of patas cells which is due to some event in the infectious cycle other than adsorption of virus (Hsiung and Melnick, 1958*a*).

Other studies of a similar nature have been carried out using established cell lines in continuous culture. Using Type 2 poliovirus it was found that resistant cells (rabbit kidney and strain L) adsorbed virus much less efficiently than susceptible cells (monkey kidney and HeLa) and that the virus adsorbed was more firmly bound to the susceptible cells and appeared to enter the eclipse phase fairly rapidly (Silverberg, Habel and Takemoto, 1959)

loosely bound, since egg adapted mumps virus binds to L cells and fails to elute, though it produces no detectable effect on the cells and does not multiply (Bader and Morgan, 1959) A systematic study of this difference in susceptibility of various mammalian cells to poliovirus confirmed the fact that a number of susceptible strains of primate cells, including HeLa, strongly adsorbed poliovirus, while insusceptible non-primate cells did not and the virus that did adsorb to non-susceptible cells eluted spontaneously (McLaren, *Holland and Syverton, 1959)* Furthermore, the virus adsorbed to the non susceptible cells failed to enter the eclipse phase Additional studies (Holland and McLaren, 1959) revealed that the capacity of HeLa and possibly the other susceptible cells to *adsorp, receive and eclipse poliovirus was dependent on the* presence in the cell wall of a specific cellular receptor which [illegible]

[illegible]

infectious virus [illegible]

virus show a sharp reduction in infection rate when treated with the receptor-destroying enzyme (RDE) In the living chick egg, these receptors are regenerated with the passage of time and susceptibility to infection returns The discovery that the mucus secreted by respiratory epithelial cells contains a material similar to the receptor substance, which may bind virus and thus render it incapable of infection, provides evidence that two host cell factors have a rôle in the determination of viral virulence, i e a specific cell wall receptor and a cell secretion

Host cell factors in viral replication

A number of investigators have reported that following the exposure of a large number of cells to a given virus which ordinarily produces a CPE on these cells, a few may survive and multiply even though virus may persist in the culture In the case of polio

not due to differences in their capacity to adsorb poliovirus but to the subsequent probability of infection and cell killing by an adsorbed virus particle

These experiments demonstrate that populations of continuous cell lines are composed of members of different

are therefore resistant

In other investigations in which large cell populations have been

that at a given time only a proportion of the cells were in the proper physiological state to undergo infection However, prolonged cultivation of the carrier cultures tended to select cells with increased resistance to Coxsackie A9 infection In studies with a *persisting infection with NDV in MCN cells, Deinhardt* and coworkers (1958) also came to the conclusion that, at any moment,

The author postulated that the presence of the serum produced an optimal physiological state of the cells in which they could carry the burden of viral infection without a CPE However, the possibility that this was due to antibody in the serum could not be eliminated even though rabbit antibody did not produce a similar

with psittacosis virus demonstrated that the maintenance of chick

penetration and development of the eclipse phase but

multiplied when the missing amino acid and horse serum were added Such omission of amino acids from the culture medium

has been shown to result in their disappearance from the free amino acid pool of the cell (Piez and Eagle, 1958)

Studies with poliovirus infection in HeLa cells (Darnell and Eagle, 1958) showed that cells depleted of vitamin B_6 failed to support viral growth and that this was due to a disappearance of certain amino acids from the free amino acid pool from which the virus was synthesized (Levintow and Darnell, 1959) Herpes virus infection was also altered in a similar manner since it infected L cells but failed to multiply when amino acids and water soluble vitamins were absent from the culture medium (Pelmont and Morgan, 1959) Type 2 adenovirus produced a more rapid destructive effect on KB cells when the concentration of arginine in the medium was increased 10 times (Bonifas and Schlesinger 1959) An increase in virulence of the virus for these cells could be produced by raising the concentration of arginine It is of interest in this connexion that Mills (1958) obtained evidence that the requirement for certain amino acids of the cells of the chorioallantois of de-embryonated eggs is increased when they are infected with the Lee strain of influenza B virus

An adequate nutritional state with reference to water soluble vitamins and amino acids is a necessary condition for the physiological competence of some cells to support the growth of certain viruses which have entered them and hence one type of intra cellular host factor determining virus virulence appears to be established

A decrease in the concentration of bicarbonate in the medium may interfere with the [illegible]

assume that this was solely an effect of pH on the host virus interaction, but adjusting the pH of the medium with NaOH did not influence plaque development with poliovirus (Hsiung and Melnick, 1958*b*) although virus growth is reduced at low pH Furthermore, in the investigations on the rôle of bicarbonate in growth of Coxsackie B-1 and polioviruses in HeLa cells the bicarbonate deficiency [illegible]

pH effect in phases of cellular metabolism necessary for poliovirus [illegible]

proteins, presumably enzymes, which are necessary for virus synthesis

Perhaps the earliest observations on physiological factors and host susceptibility to virus infection *in vitro* are those of Enders and Pearson (1941) who reported that tissue cultures of chick embryo lung supported the growth of the Melbourne strain of influenza A virus at 37° but not at 41°, though other influenza virus strains grew at this temperature (Colville, Dunbar and Morgan, 1956) Growth of polio virus has also been shown to be reduced at a temperature of 39° (Lwoff and Lwoff, 1958) Wheeler (1958) showed that a change in the temperature of incubation of the host cell from 28° in successive steps to 40° had a significant effect on the multiplication of herpes virus in HeLa cells with a maximum virus yield at 35° Furthermore, cells incubated at 28° and showing little or no evidence of virus reproduction promptly produced increased amounts of virus when the incubation temperature was raised to 35° In other studies with influenza virus it was shown that at 25° the virus did not grow in the cells of the allantoic sac of the chick embryo, but *soluble antigen and haemagglutinin accumulated in* the cell and transfer of the infected eggs to 37° led to a rapid appearance and release of infectious virus (DeSanctis and Liu, 1959) The temperature of the host cell therefore is an important factor in viral replication, and if it is low, though virus may undergo incomplete replication, it does not attain the infectious form and therefore fails to manifest virulence

limited replication of the virus which in most instances destroyed the cell However, all cells infected and undergoing this partial growth cycle were not destroyed A similar situation has been described with NDV infection of Ehrlich ascites tumour cells in which the virus penetrated the cell but failed to complete its growth cycle though the cell was destroyed (Prince and Ginsberg 1957) In these instances some host cell factor prevents completion of the growth cycle and thus influences viral virulence, for even if the cell is destroyed the viral components released are not infectious and therefore not virulent

Other viruses such as mumps may destroy cells *in vitro* without detectable viral replication (Henle, Deinhardt and Girardi, 1954)

Morgan, 1950) This viral property which may be related to virus virulence (Morgan, Soule and Marinetti, 1959) is dependent on host cell factors for its manifestation

Host cell factors and tumour viruses

Before concluding this discussion, it is important to consider host cell factors that influence the virulence of tumour viruses, for in this case virulence is not manifest *in vivo* as a destructive action on host cells (with the exception of the haemorrhagic disease produced in chick embryos by Rous sarcoma virus) but rather as their conversion into a new host cell virus system which perpetuates itself and is associated with unrestrained multiplication of the cells *In vitro* however the tumour viruses may demonstrate either the capacity to transform normal cells to a malignant state or to produce a CPE on the same cells An analysis of this pheno

subcultures could be made from these degenerating cultures and vigorously growing, apparently healthy cell colonies were obtained which continued to produce Rous sarcoma virus, but these in turn

degenerated None of the cells demonstrated an increased proliferative capacity. It was later discovered (Temin and Rubin, 1958) that if the chick embryo fibroblasts transformed by Rous sarcoma virus were maintained under better nutritional conditions, the transformed cells did not show a CPE, but multiplied and could be grown for twenty generations Thus, the physiological state of the host cells determined their response to the virus, i e

It was also discovered that at any one time only 10 per cent of the chick embryo cells were susceptible to infection by Rous sarcoma virus even if a clonal population was used, and the investigators believe that the physiological state of the host cell is of importance in deciding whether or not it is competent to be infected by Rous sarcoma virus (Temin and Rubin, 1958)

In the case of Rous sarcoma virus, the physiological state of the host cell may determine whether or not the cell can be infected and which two diametrically opposite results i e cell death or cell multiplication, will occur

DISCUSSION

Isaacs One of the factors in cellular resistance may be the induc-

due to the production of a substance which they ca[illegible] inhibitory factor) That substance seems to be very similar in nature [illegible] stently nfected

multiplying?

Morgan No

Isaacs They produce virus without multiplying?

Morgan These cells so far, do not multiply in such a simple synthetic medium without the introduction of serum I have never

yet been able to make the cells multiply without the production of virus These cells depleted of a certain single amino acid will remain

out a long time ago the case of pneumococci and their pathogenicity for the rabbit—that you could correlate this very neatly with

[illegible]

Morgan If you select out the heat-resistant variant you will get a pathogenic effect also at 40°.

Andrewes Dr Isaacs has suggested that in tissue cultures of [illegible]

the interferon theory

[illegible]

used

Kilbourne Is it clear that the virus is intracellular in those experiments?

Morgan It is probably multiplying at or near the cell surface

Burnet Did those resistant cells maintain their resistance on further cloning?

Morgan I think so

Burnet This would be crucial for deciding whether it is a physiological or a genetic variation

[illegible]

particle At any rate, under these circumstances found in the infected, depleted cell, for a period of three weeks nothing is happening in those cells except perhaps the replication of these large bodies Now if they were multiplying we ought to see them because these are easy to recognize in the electron microscope

carrying out over several years, and more recently in tissue culture material, I have noticed a very constant relationship between the nucleolus and the nucleus during the stage preceding what is usually regarded as "cytopathic" change, nucleolar enlargement being the

along those lines At the submicroscopic level a vast amount of material would have to be examined before one could be certain whether or not significant changes exist in such altered cells

Morgan Alterations have been reported in some of the carrier cells, with the poliomyelitis virus, in which you can produce the cell carrier state These cells are altered in that even though they

influenza virus

Pereira When one considers the parallelism between virulence

pathogenicity for man Types 1 2 5 and 6 are the viruses which most frequently cause latent infections whereas Types 3 4 and 7 are the ones more commonly associated with epidemics in man These two groups formed on the basis of pathogenicity for man can also be grouped by several laboratory properties and an investigation of possible reasons for this grouping might lead to some interesting

Pereira In that respect taking the adenoviruses the type of cytopathic effect of Types 2 5 and 6 for instance is remarkably dif

THE EFFECT ON VIRULENCE OF CHANGES IN PARASITE AND HOST

C. H Andrewes

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Nowadays we all accept Theobald Smith's dictum that the natural tendency of a host-parasite relationship is towards a mutual tolerance which will permit a survival of both partners True, the association is at times an uneasy one, liable to be disturbed by factors of various kinds Rather than dwell upon this, however, let us consider the cases in which some degree of virulence favours survival of a virus, by facilitating its spread to fresh hosts

Respiratory viruses

Viruses which are spread from the respiratory tract would seem to have a better chance if the infected host coughs, sneezes or otherwise distributes excessive secretions A measles or a common cold virus which was completely avirulent and caused no coryza would be expected to spread very poorly This sounds entirely reasonable nevertheless it is not sure that this is always true In one experiment at Salisbury on transmission of the common cold by contact, the only cold amongst the recipient normal volunteers was in one exposed to an infected volunteer in the incubation period of his cold and as yet without any symptoms On the other hand, influenza spreads very successfully only when it is fairly virulent We know that inapparent immunizing infections occur, [illegible] low, [illegible] We [illegible] and sneezing almost certainly helps the spread of some respiratory viruses, even though others can spread at times without the aid of this expedient

Enteric viruses

The many viruses causing diarrhoea—in man, mice, pigs or cattle—are all, so far as we know, excreted in the faeces, they surely

have a better chance to spread because of the greater volume of excreted faecal matter which results from their virulence for the intestinal tract On the other hand, the enteroviruses proper—the polio, Coxsackie and Echo viruses—seem to spread quite readily, though commonly causing no diarrhoea at all If we belong to a school of thought which believes in their spread from the respiratory tract, it is then relevant that except for the herpangina caused by some Coxsackie viruses, enteroviruses do not produce symptoms at that end of the host either The virulence they show for the nervous or muscular systems seems to be accidental and quite irrelevant to the virus's evolutionary needs These viruses are all tough, resistant to many physical and chemical agents, more so than the respiratory viruses (other than the adenoviruses). So perhaps they can persist in the environment long enough to have a reasonable chance of reaching a new host they have not needed to acquire or retain the virulence necessary to aid their spread A parallel is afforded by ticks and trombiculids which can afford to wait hungrily for prey for weeks at a time, they do not need wings like the more delicate mosquito One might forecast for the enteroviruses that, with improvements in hygiene, things will get harder for them and that they will have to evolve a more efficient mechanism for distribution, even that spread from the respiratory tract might prove to be operationally more worth while than reliance on a faecal route

Arborviruses

The situation with arborviruses is very different here, the important point is the height and duration of viraemia, giving optimal chances of spread by an arthropod to another host Most arborviruses probably inhabit a reservoir host for which they are harmless this fact speaks against any evolutionary tendency towards higher virulence for the host to aid spread Assuming that monkeys constitute the natural reservoir of jungle yellow fever in Africa, we may note that the virus, which is normally harmless to them, perpetuates itself just as readily as it does in South America where the local monkeys tend to suffer from lethal epidemics Macnamara (1954) found that clinical severity of yellow fever in man was not correlated with duration of viraemia Virulent infections by the equine encephalomyelitis viruses in horses and man are certainly of no value to the virus, they are blind-alley infections

with a viraemia too short and too low to help the virus to spread One cannot conclude, however, that spilling over of arthropod viruses into strange hosts is never associated with a high viraemia leading to rapid epidemic spread in the new host. This is doubtless what has happened in the past when jungle yellow fever has led to an *Aedes aegypti–Homo sapiens* cycle, very favourable to the virus With certain apparently new epizootics caused by arborviruses, one may suspect that something similar is happening

Before dismissing virulence as unimportant for the survival of arborviruses we must recall that it can be looked upon either from the point of view of the whole animal, as a factor leading to illness or death, or on a microscale as a matter between the virus and the parasitized cell—a sort of microvirulence If the cells are of an expendable sort this may matter little to the host At this cellular level, however, it may be that a more effective microvirulent virus may be one which leads to higher and longer viraemia and prospers in consequence.

Myxomatosis

We may speculate about the effect of natural selection on the virulence of other viruses, but about myxomatosis of rabbits we have actual knowledge. The facts are of much interest Natural selection appears to be acting to favour a virus, not the most or the least virulent, but one having an intermediate, optimal virulence

In Australia, spread is almost wholly through the agency of mosquitoes, and these, to be efficient vectors, must pick up virus by biting through highly infectious skin lesions, not enough virus can be acquired from blood Since 1950–51 the virulence, as judged by mortality rate, has been falling Fenner and Marshall [illegible]

after a standard inoculum, they estimated this latter value, therefore, and could then classify their virus strains into any of 5 grades of virulence The evolutionary tendency towards a less virulent virus is explained by the usefulness to the virus of longer survival of rabbits in a state capable of transmitting infection to fresh hosts through mosquito bites The effectiveness of the selection is shown by the fact that highly virulent virus has been introduced into areas where the virus is less lethal than Australian farmers would like;

yet in a matter of months the older, milder strains have completely pushed out the more rapidly fatal ones The loss of virulence must not, however, be carried too far, strains whose virulence is too low fail to give rise to a good enough lesion or this regresses too early, so that mosquitoes acquire virus less readily

In Britain, also, attenuated variants have appeared but they have not been replacing the virulent strains as rapidly as in Australia It appears, in fact, that the ratio of virulent to attenuated strains may in places be increasing The differences may be due to the fact that the main vector in Britain is the rabbit flea, not the mosquito Now the Australian rabbit, infected with an attenuated virus, lives for many days and so can infect many mosquitoes which bite it when its tumours are full of virus, fly away and later bite and infect another rabbit The British rabbit flea, which is a rather sedentary species has, on the other hand no temptation to leave a mildly infected rabbit. If this dies after a chronic illness, there may be but little virus, or virus mixed with antibody, on the flea's proboscis, and if the rabbit recovers there may be no virus at all

strain

survive to keep the infection going If less virulent virus is also present with it, numbers of rabbits will recover and their progeny, fully susceptible, will be on hand to help perpetuate either virulent or avirulent strains It is hard to forecast how natural selection might act upon such an intriguing and complicated situation, perhaps it might favour a virus which as regards its virulence was genetically unstable

mortality was down after 4 or 5 years to 50 per cent, while after 7

annual epizootics only 30 per cent of inoculated wild rabbits died An interesting result of this change is foreseeable virus of optimal virulence for the unmodified rabbits may be too attenuated to have the best chance of survival in the rabbits of today Virus may therefore tend to attain not to a fixed level of virulence but rather to operationally similar behaviour this in practice will mean a more virulent strain assessed in terms of tests on unmodified domestic rabbits

The level of virulence of other viruses at large today may well be the result of interplay of similar factors The low virulence of measles virus for Europeans as compared with that of virg n populations as in Fiji and the Faröes may be due partly to genet c influences and partly to factors concerned rather with immunology

Rinderpest

Another example of genetic differences in resistance to a virus is worth mentioning because it has such a practical bearing on efforts at control by immunization Rinderpest virus has been attenuated in the laboratory in three ways—by passage through goats through rabbits and through chick embryos In each instance the virus has gained virulence for the new host but has become more or less attenuated for cattle The precise level of attenuation which is best for practical use depends upon the breed of cattle concerned (Brotherston 1955 56) A vaccine which is excellent for many [illegible]

highly attenuated for the more resistant zebu and the still more re sistant buffalo and will have little useful immunizing effect in them It seems not unlikely that the zebu and buffalo coming from countries where they have had to coexist with rinderpest for many years have acquired some genetic resistance just as have the Australian rabbits to myxomatosis

Summary

Multiplication and spread of a virus are favoured if it is not too virulent so that the invaded host survives for a reasonable time to furnish a source of infection of fresh hosts On the other hand a certain degree of virulence either at the level of the whole host or of the cell may be necessary to provide [illegible] tre in blood or

excretions, so that there is plenty of virus available for a new host The most successful virus will therefore have the optimum virulence for a particular host But since the host's resistance may itself change through the operation of genetic or immunological factors, the virus may have to change too, and there results an unstable balance An excessive swing may lead to a temporary eclipse of the virus on the one hand or to an alarming epidemic on the other

DISCUSSION

Niven Dr Andrewes rarely speaks without producing some telling phrase, and the one which I liked on this occasion was 'expendable cells"

- -

between rabbits. Bull, in his early work, thought that this was probably how it would spread in the field, and from about 1933 until 1950 the standard strain was passed by cage contact between rabbits Fenner has always used that strain as control, and we can be quite

large experiment

epizootics the whole situation has been dominated by what Fenner

affecting infectivity by causing a more severe or a less severe local

effect on the rabbit virus

Dick Another important point, in relation to myxomatosis

not predict from laboratory tests of the virul-

munities

Burnet It is the integration of the whole ecological field which will termine what survives.

Another point of some interest in regard to the Australian myxomatosis experience is the nature of the process by which a relatively less virulent strain can emerge, presumably as a mutant, from a population of highly virulent material Fenner and Day have discussed this, and their conclusion is that the average dose transferred

virus you recover here is much more virulent than those in Australia It is surprising that seemingly, even if British virus were assayed in British wild rabbits and Australian virus in Australian wild rabbits

that in areas where the temperature is higher the mortality is lower, in accordance with Thompson

Kilbourne Dr Andrewes idea that a certain amount of virulence is necessary for dissemination of virus is a fascinating one We have done some experiments recently

interesting thing is that if one takes what is anti strain which is not mouse adapted, such

[illegible]

his strain of virus, and reproduced exactly the kind of cage that he used, but I just could not reproduce his results under British conditions I never found out the reason for the differences

Kilbourne We have succeeded thus far in all three experiments using the mouse-adapted strain Our test for the presence of infection in the cage mate—the contact mouse—is the presence of [illegible]

Kilbourne Some of the titres obtained are 10^7 or more

Henderson How many transfers have you obtained in this way?

Kilbourne Our test for the presence of infection has been the palpable demonstration of virus in the contact mouse lung

Henderson You have not tried to transmit this cross-infection to yet other normal mice?

Kilbourne No, not yet

Henderson In our experiments this can happen in a very small percentage of cases giving quite high virus titres, in the lung, but on [illegible]

know

Henderson One possible explanation for the lack of sequential transfer is found in the type of experimental condition that we have been using The infection is induced first of all by very small particles —clouds—in the lung and we know that the particles that come out of the mouse whenever it sheds the virus are quite large and that the chances of getting to vulnerable sites are much reduced This also happens in guinea pigs with other infectious agents

BROAD ASPECTS OF THE PROBLEM OF HUMAN VIRULENCE IN INFLUENZA VIRUSES

J. MULDER

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IN 1953 Burnet described in his book "Natural History of Infectious Diseases" what he thought would probably occur should a new pandemic of influenza appear, noting that it was difficult to write about influenza without indulging in speculation. Two years after the appearance of the 1957 pandemic, we can state that nearly every point which Burnet raised has been consistently proved correct. Indeed, one of his remarks concerned the possibility that antibiotics might be relatively useless in saving many lives. This point will be the main subject of the present paper because we believe that in 1957 a number of patients died from pure influenza virus pneumonia.

say, intrinsic bronchotropic virulence. In mice, bronchotropic virulence (Straub, 1937) of straight egg-line virus can be demonstrated microscopically in the first or second passage without coexistent damage in the lung tissue (Fig. 1, Table I). It is, however, probable that viral "toxic" lesions can appear in the ciliated epithelium in mice when a high dosage of egg-line virus is inoculated (Table II, Mulder and Hers, 1959). Studies on the individual human disease

determined by (1) the degree of intrinsic bronchotropic viru

Table I

DEVELOPMENT OF BRONCHOTROPIC AND PNEUMOTROPIC VIRULENCE IN MICE [STRAIN A1 (1951)]

(LIVERPOOL TYPE, P PHASE)

(Passage 0 = egg fluid amn 2–all 2–amn 2)

Inoculum amniotic fluid F.A.D	*Lung + amniotic passage number*	*Microscopical bronchiolar cell damage** (3 days)	*Microscopical lung tissue damage** (3 days)	*Mortality†* (*within 10 days*)
32	0	0	0	0/4
32	1	++++	0	0/4
32	2	++++	0	0/4
32	3	++++	0	1/5
32	4	++++	++	0/4
32	5	++++	+++	4/4

* Specific viral cell lesions and lung tissue damage (2 mice)
† No microscopical control.
F.A.D = dosage of chicken cell haemagglutinating units

Table II

(Preliminary experiment)

TOXIC (?) BRONCHOTROPISM AND 'TOXIC' PNEUMOTROPISM IN MICE [STRAIN A2 (1957) (P PHASE) (egg fluid amn 4)]

Inoculum E.I.D	*Microscopical bronchiolar cell damage** (2 5 days)	*Microscopical lung tissue damage** (2 5 days)	*Mortality†* (*within 10 days*)
10^8	++++	++	1/2
10^7	++++	++	1/2
10^6	++++	+	1/2
10^5	+++	0	0/2
10^4	±	0	0/2
10^3	0	0	0/2

* Specific viral cell lesions and lung tissue damage (1 mouse)
† No microscopical control.
E I D = egg infectious dose

of the virus (ii) the size of the infecting dosage, (iii) the immunological resistance of the patient and the human herd, (iv) host factors of unknown nature (perhaps associated to some extent with age), and (v) unknown climatic factors (Andrewes, 1959)

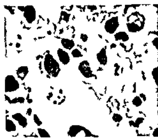
The apparently stepwise increase in extent and severity of epidemics of influenza A1 observed in many countries in the period



FIG. 1 Section of mouse lung 2 5 days after intranasal infection with 32 F A D of the first mouse egg passage of a lung non-adapted influenza A1 (1951) virus (Liverpool type P phase) Complete cytonecrosis of bronchiolar epithelium The lung tissue is normal and all control mice survived (*see* Table I) oil immersion



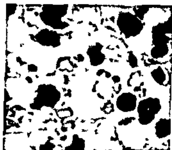
(*a*)



(*b*)

FIG 2*a* and *b* Section of mouse lung 3 days after intranasal infection with lung adapted influenza swine A virus (allantoic fluid) Broadening of alveolar septa with interstitial exudate

Cytopathogenic changes in what are believed to be alveolar cells with nuclear fragmentation and vacuolization oil immersion



(a)



(b)

nuclear fragmentation o l immersion

1946–1951, after the appearance of the antigenic variant A1 (Stuart-Harris, 1953) may perhaps be explained by a periodic increase in intrinsic bronchotropic virulence of successful A1 strains, and the same may have occurred after 1934 when the

ferret This is clearly demonstrated by the different responses caused by the viruses of influenza A and B Careful comparative virological and histopathological studies within the groups of influenza A and B regarding their initial intrinsic virulence for the ciliated epithelium of the respiratory tract of ferrets and mice are, as far as we know, lacking Titrated original infective human material will also be needed for experiments of this kind because egg-passage may lead to less pathogenic artifacts, as has been clearly demonstrated by Burnet and Bull (1943) in their work on OD-phases in former human A-strains and in vaccination experiments with intranasal application of straight egg-line virus (Isaacs and Roden, 1956, Isaacs, Negroni and Tyrrell, 1957)

PQ-phase differences may also be related to the problem As far as we know, however, it is not certain that both phases actually occur in the human disease Q-phase egg-line virus is less antigenic

hemisphere existing exclusively in the P-phase (Isaacs, Gledhill and Andrewes, 1952), and Smith, Westwood and Belyavin (1951) have demonstrated an abnormally virulent or "toxic" behaviour

(Fukumi, 1959) Nothing is yet known about their intrinsic bronchotropic virulence in ferrets and mice in comparison with

To summarize, we may conclude that differences in the degree of intrinsic human bronchotropic virulence in influenza viruses within the A- and B-group quite probably do exist, but more satisfactory standardized laboratory methods are needed to enable us to determine this quality more quantitatively, assuming that the experimental animal is a reliable test object

Still more difficult is the situation concerning the problem of human pneumotropic virulence of influenza viruses Before 1957 we had only the vaguest idea about the possibility of the existence of human influenza-virus pneumonia, which emerged exclusively from the experience of pathologists during the catastrophe of 1918 and from the knowledge that most strains of influenza virus A can be made killers of mice, ferrets and hamsters, causing the well known swollen, plum coloured consolidated lungs Naturally occurring swine influenza can also cause virus pneumonia in this animal

On examination of 148 virologically proven fatal cases of Asian influenza in the Netherlands, Dr Hers and I came to the conclusion that about 20 per cent had died from a curious form of non-bacterial pneumonia which resembled closely some of the accurate descriptions of 1918 influenzal pneumonia given by Le Count [illegible] 1959, Newcombe, Nixon and Thompson, 1958, Rock, Braude and Moran, 1958) In the period 1941–1957, Hers (1954) examined *18* [illegible] in the trachea and, in the majority of cases, to extend down to the interlobular bronchioli but only very rarely to the respiratory bronchioli, and probably not to the alveolar ducts *No evidence of* virus pneumonia was present All cases, except the one infected with influenza B, died from coexistent bacterial bronchopneumonia A similar type of lesion was found in 66 fatal cases of *secondarily* infected Asian influenza as well as in 6 cases which died *from other* causes during the acute phase of this disease

In 30 other fatal cases of Asian influenza without coexistent bacterial inflammation the epithelial lesions were found in the respiratory bronchioles and alveolar ducts, and heavy damage had been caused in the alveolar septa In the Netherlands, the incidence of this fatal type of pneumonia has been very low and can be roughly estimated to have been 1 in 4,000 cases of Asian influenza including cases with coexistent bacterial infection There is strong evidence that experimental influenza-virus pneumonia in mice, ferrets and swine is basically the same pathological process as in man, but it is more difficult to demonstrate capillary thrombosis in the animal disease. However, typical cytopathogenic changes in what is most probably alveolar epithelium with fragmentation of the nuclei can be clearly observed (Fig 2a and b) It is a remarkable fact that we are learning to understand experimental influenza-virus pneumonia from the autopsy findings in the human disease It is probable that the capillary thrombosis with interstitial focal leucocytic exudate, capillary bleeding and "hyaline membrane" formation seen in human influenza-virus pneumonia are phenomena which are secondary to the necrosis of the alveolar lining, but the possibility that the virus can also damage the capillary endothelial cells cannot be refuted In the near future many new details of influenza-virus pneumonia in both man and animals may be found by applying the technique of fluorescein-labelled antibody and by electron microscopy. On indirect evidence, however, it is reasonably certain that we can define pneumotropism of the influenza virus as its potency to multiply in the alveolar lining with subsequent destruction of the alveolar cells. Through the work of Burnet (1954, 1955) we can postulate that, during the process of adaptation in the animal lung, the virus develops new genetically determined qualities to destroy this new type of cellular host

We may conclude that human influenza virus pneumonia occurred in the 1918 and 1957 pandemics and that the lung damage in man is basically identical with the experimental virus pneumonia in susceptible animals.

The first question we must ask is whether the various strains of [illegible]

again under the influence of a new great pandemic, the same occurred but there is no certainty that diagnostic efforts were stimulated on a sufficient scale between 1919 and 1957 or that it was considered worth while in this period to publish findings identical with those seen in the pandemic year of 1918

The negative findings of the 18 carefully studied cases in Leiden between 1941 and 1957 offer no convincing evidence in comparison with the 148 cases studied in 1957. There has been one case, published by Parker and co-workers (1946), in which there most probably occurred a pure influenza virus pneumonia This was a case of influenza A from the year 1943, caused by a PR8 type of strain

Perhaps there is one clue to an answer, relating to the fact that, in 1957, influenza-virus pneumonia often occurred in patients with chronic heart disease, including chronic rheumatic endocarditis In the Netherlands Dr Straub, a pathologist who has been a keen observer of fatal acute lung disease in Rotterdam, made a careful study of the literature on 1918 influenzal pneumonia and found to his surprise that early and late in this pandemic four publications by pathologists (Glaus and Fritsche 1919, Oberndorfer, 1918, de Vries, 1919, Wegelin, 1919) mention the relatively high frequency of coexistent chronic endocarditis, especially mitral stenosis (Straub, 1959) However, neither in his nor in our experience has chronic endocarditis been a clearly defined underlying condition in influenzal pneumonia during the period 1937–1957 Semple (1959) has found no significant increase in the death rate from mitral stenosis or chronic endocarditis during the severe Liverpool epidemic of 1951 On the other hand, it should be emphasized that the fatal case of influenza-virus pneumonia described by Parker and co workers (1946) concerned a 39 year-old man who had myocardial fibrosis and was an iron worker, both conditions perhaps favouring the development of this disease

In conclusion, we can say tentatively that in the period 1933–1957 the human strains of influenza virus did, but only very rarely, cause virus pneumonia in contradistinction to the pandemic 1918 strain and to the Asian type

What can experimental virology contribute to the understanding

of the problem of human influenza virus pneumonia? It has been shown by Shope (1935) that a laboratory strain of the swine A-virus is pneumotropic in mice and ferrets in the first passage and remains so in the following passages This indicates that there might also be a chance that enhanced pneumotropic virulence of strains circulating in human beings can be demonstrated experimentally although, at present, human host specificity cannot be excluded The Asian virus isolated from sputum or emulsion of lung tissue from fatal cases of virus pneumonia needed a certain number of passages to become fully adapted to the mouse lung, and did not differ in this respect from many former A-strains In 6 out of 7 cases tested, Asian virus from human sputum or lung tissue could be established directly in the lung of mice, but the same was observed by Francis and co-workers in 1937 when the A-PR8 virus was prevalent, and it should be emphasized that the Asian virus is non sensitive to the neutralizing factor present in normal mouse serum Medill-Brown and Briody (1955) have shown that certain non-adapted egg-line strains which are made non-sensitive to this factor can more easily and more rapidly be adapted to the mouse lung than the original sensitive parent strains In our tests, using the crude method of passing 10 per cent emulsion of lung tissue, fatal virus pneumonia did not develop in mice before the fourth passage and there was no difference in this respect between strains isolated from sputum of uncomplicated cases and strains isolated from lung tissue of fatal cases of virus pneumonia Many isolates from cases of human virus pneumonia were Q phase strains in contradistinction to the observation that when Q-phase egg-line virus becomes adapted to the mouse lung, a change to P-phase strains invariably occurs. We have so far not carried out careful comparative work, but it should be noticed that investigations of this kind should include former A strains which are previously made non-

In itself, the phenomenon of the egg-lines of the Asian virus being non-sensitive to this factor, first focused by Isaacs (1959) is

adapted lines are for the most part non sensitive (Chu, 1951, Brans, Hertzberger and Binkhorst, 1953) We have thought for a while that, in general, non-sensitive A-strains might have a greater intrinsic human pneumotropic virulence than sensitive strains Human serum, however, possibly does not contain the neutralizing factor (Chu, 1951) and the change from sensitivity to non sensitivity during the adaptation in the mouse lung may also be caused by selection of non-pneumotropic, non sensitive mutants, causing a greater survival chance of the resistant variant in the mouse lung (Medill-Brown and Briody, 1955)

A complex but very interesting phenomenon in the problem of experimental influenza-virus pneumonia is the so called "toxic" or "non-transferable" pneumonia which can appear in mice and hamsters when inoculated with live, high titre, complete egg-fluids of lung non adapted virus (Anderson and Burnet, 1947, Burnet, 1955, Dudgeon *et al*, 1946, Sugg, 1949*a* and *b*) We found to our surprise that this pneumonia shows histopathologically very nearly the same picture as that caused by fully lung adapted strains (Fig 3*a* and *b*) In the light of recent experience it is probable that this pneumonia is the result of viral destruction of the alveolar cells caused by a multiplication to incomplete virus [illegible]

shown that toxic pneumonia can be provoked in mice with a lower dosage of lung non-adapted A1 egg-line virus (allantoic fluids) that is previously made non-sensitive to the neutralizing mouse serum factor What precisely is the underlying mechanism in this type of (cell) injury is unknown, but it should be emphasized that when a great quantity of lung non adapted influenza virus reaches the alveoli, a fatal pneumonia may appear which so far cannot be distinguished microscopically from the pneumonia caused by fully lung-adapted strains Straight, high titred egg-line fluids of the Asian type also show the phenomenon, but we have not yet performed a careful quantitative comparison with former human A-strains which previously have been made non-sensitive to the neutralizing serum factor The problem is difficult because viral "toxicity" may be enhanced by a certain degree of genetically determined pneumotropic virulence

It may be asked whether the lung damage from the 1957

pandemic is perhaps related to this toxic pneumonia in experimental animals Kilbourne (1959) has shown that human lung tissue from fatal cases in this pandemic has yielded amniotic titres in a dilution of 10^{-8}, and we know that in fatal virus pneumonia the epithelium of many respiratory bronchioli and alveolar ducts are destroyed by the virus Thus it is conceivable that mass aspiration of virus in the alveoli may occur

This speculation of a possible relationship of 1957 human influenza-virus pneumonia to experimental toxic pneumonia is better suited to the observation of the low incidence of virus pneumonia in previously healthy people than the assumption of an increased degree of human intrinsic pneumotropic virulence of the Asian virus In the latter case we would rather have expected a mortality or morbidity of the 1918 type

The problem of human influenza virus pneumonia cannot be dis-

logical resistance in most individuals, thus causing the extensive and rapid spread *It is quite possible that former human A-virus* appearing after 1918, even when it reached the lung in sufficient quantities to cause lung damage, was often counteracted in the lung tissue by specific antibody provoked by dominant or shared minor antigen present in precursor strains This speculation is amenable to an experimental approach

As in most clinical problems, host factors have played a distinctive rôle in human Asian influenza-virus pneumonia Whole series of clinical and fatal cases have been observed in North America (Louria *et al.*, 1959, Rock, Braude and Moran, 1958), in England (Giles and Shuttleworth, 1957 Newcombe, Nixon and Thompson, 1958) and in the Netherlands (Hers, Masurel and Mulder, 1958, Straub, 1959) in pre existent chronic congestion of the lungs associated with chronic heart disease (especially valvular defects), certain chronic lung disease and pregnancy The explanation of this coincidence is not clear In chronic congestion of the lungs two factors might have been responsible First, the elimination of virus from the lungs might have been hampered by the chronic congestion in blood and lymph vessels, thus causing an accumulation of a high concentration of the virus and, secondly, the hyperplasia of the alveolar cells commonly seen in chronic congestion

the lungs might have made these cells more susceptible to viral multiplication or to "toxic" viral destruction The problem might perhaps be studied in ferrets or pigs, in which chronic congestion of the lungs has been established experimentally.

In summary, we would tentatively conclude that 1957 human, influenza-virus pneumonia was not caused by strains with a high degree of human, intrinsic pneumotropic virulence but that the lung damage was caused (i) by mass aspiration of virus, (ii) by lack of protecting antibody, and (iii) often by certain unknown, pneumotropism-enhancing host factors, especially chronic congestion of the lungs If human serum or lung tissue should contain non-specific neutralizing substance the non sensitivity to it of the *Asian strain might have contributed to its pneumotropism*

We feel that the experience gained from the 1957 pandemic throws more light on the mortality in the 1918 pandemic. We may assume that in 1918 the human population was also deprived of

thus showing that influenza-virus lung lesions played a substantial rôle in causing mortality, which was also enhanced by secondary bacterial infection Coexistent chronic endocarditis was found in relatively high frequency by pathologists working in general hospitals The high mortality throughout the world population in the second wave suggests a certain degree of human intrinsic pneumotropic virulence of the causative virus, which may have been enhanced in October and November 1918 and by a host factor associated with the 20–50 age group

Evidence now being accumulated (Davenport, Hennessey and Francis, 1953) makes highly probable the hypothesis of Laidlaw (1935) and Shope (1936, 1958) that the virus of swine influenza was the cause of the 1918 pandemic In this respect it would be very worth while to study freshly isolated field strains of swine *A virus* and to compare their degree of pneumotropic virulence with former human A-strains and the Asian virus, because these strains, which are antigenically closely related to the first isolated swine virus and can cause virus pneumonia in swine, may show a higher degree of initial pneumotropic virulence in ferrets and mice

The present paper, including more questions than established facts, has been born out of some disillusion which we have ex- [illegible]

[illegible]

which has contributed to the saving of thousands of human lives, it was not feasible in 1957 to predict on sound grounds the possibility of the appearance of a most dangerous complication of the disease, namely, human influenza-virus pneumonia

DISCUSSION

Morgan You referred to the incidence of fatalities in patients with heart disease did you have evidence of an influenzal myocarditis in any of these patients ?

[illegible]

pneumonia

Morgan Do you accept any of the other described cases of influenzal myocarditis ?

Mulder [illegible]

whether in the case reported it may have been a pre existing event which in fact contributed to the development of the viral pneumonia

Morgan What about the reported isolation of influenza virus from the myocardium ?

[illegible]

myocarditis was not proved to have been present. At the same time [illegible]

[illegible] these patients The mortality was seasonal, irrespective of the epidemic These are two almost contrary experiences in the same epidemic heart cases in some curious way seemed to be badly hit, whereas the ordinary patient with chronic bronchitis and emphysema was not so badly affected.

Morgan Have you done any electrocardiographic studies in patients whom you thought had influenzal pneumonia to see if there was evidence in those that survived that they might have had myocarditis?

Stuart-Harris · Not serially

[illegible]

higher pressure curve in the lung circulation, a very high PCO_2 in the peripheral blood and a right preponderance I think there is in all [illegible]

* Not reproduced in the present volume—Eds.

myocarditis was not proved to have been present At the same time on my ward we had eight cases of influenza in patients with rheumatic heart disease of various kinds We have never had such numbers during any previous influenza epidemic but as none died there was

experience In the fairly large vaccination trial which we carried out in patients with chronic bronchitis during that winter we did not find that the Asian epidemic produced a peak in mortality amongst these patients The mortality was seasonal irrespective of the epidemic These are two almost contrary experiences in the same *epidemic heart cases in some curious way seemed to be badly* hit whereas the ordinary patient with chronic bronchitis and emphysema was not so badly affected

Morgan Have you done any electrocardiographic studies in patients whom you thought had influenzal pneumonia to see if there was evidence in those that survived that they might have had myocarditis ?

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higher pressure curve in the lung circulation a very high PCO_2 in the peripheral blood and a right preponderance I think there is in all

* Not reproduced in the present volume—Eds

before gross alterations appear in the nuclei, but have not so far carried out a sequential systematic investigation of the cytochemical changes

stage but it is tempting to speculate on whether or not they may represent distinctive strain differences in an indicator cell system which might have relevance to a study of virulence

Mulder Our problem is at present mainly topographical Of course, most of the bronchial cells are degenerated or necrotic,

Hers The histopathological study of influenza virus pneumonia

inoculation

Kilbourne Dr Hers, have you correlated the existence of the so-called hyaline membrane with the presence of these changes in th

alveolar cells? In other words, have you seen changes of this type in the alveolar cells in the absence of hyaline membrane?

Hers Yes, I have The membranes are a late phenomenon

Kilbourne This is a question of the specificity of the lesion because you can see it in hypoxic conditions in infants One wonders whether it is much to lean on in terms of assessing specific damage to the cells by influenza virus

Hers I think the hyaline membrane production is non specific and only a late phenomenon as a consequence of the de-epithelisation of the alveolar wall

Burnet I got the impression that you did not consider the ability to produce viral pneumonia (specific alveolitis) as a genetic characteristic of the 1957 strains but rather a result of complete lack of antibodies in the persons concerned Is that correct, or do you think that the virus has a specific genetic character, the ability to attack human alveoli? I am interested because a specific change in antigenic character took place in 1946–47, which was not associated with the appearance of alveolar virus pneumonia It would seem that the 1918 and 1957 strains may have had a genetic character of being able to produce this alveolar attack in a proportion of human cases The only two years for which there is evidence are 1918 and 1957 Do you think that there is a specific genetic difference which separates those strains from the new A1 strain?

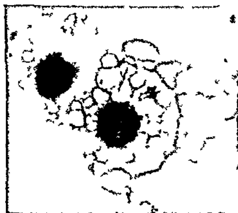
Mulder This is still a matter for speculation With crude methods we do not find any difference with former A strain in relation to animal pneumotropism We might accept that many people aspirate virus during the acute disease Since we have had a mortality from influenza A_2-virus pneumonia of 1 in about 4,000 cases it is very difficult at present to accept a genetic enhanced pneumotropic virulence Therefore, we concluded that in some people mass aspiration might have taken place which might cause the same anatomical picture of pneumonitis But that is also a speculation On the other hand, in 1918 the epidemiological picture was quite different The mortality in some regions was 1 per cent It is difficult to deny that the 1918 virus had no enhanced intrinsic pneumotropic virulence Therefore, I should like to see how freshly isolated swine A-virus behaves I have the impression from the work of Shope that it goes very rapidly in mice, so that these strains might have retained the original pneumotropic virulence But why did the former human A strains, which probably are derived from A swine, fail to attack the lung? Is the lung protected by antibody, or is pneumotropic virulence lost by the continuous circulation in the bronchial epithelium? We know that when lung adapted strains are passed in eggs, they may lose some virulence for the lung tissue

Burnet There was some evidence that the 1918 strains lost virulence within the next few years When New Caledonia received the 1918 strain for the first time in 1920 it was much less virulent

Mulder There may be several reasons First, the circulating strains in this period may not have been alike Secondly, strains may



FIG 1 (Hers) Fluo esce n labelled ant body techn que app ed to c olog cal smears of nfected mouse lung Spec fic fluorescence n cytoplasm of 2 p obably al eol ce ls o l mmers on



F G 2 (Hers) The same cells as n F g 1 res a ned w h haema oxy n eos n One cel shows an ab normal acuol zat on o l mmers on

THE SEVERITY OF INFLUENZA AS A RECIPROCAL OF HOST SUSCEPTIBILITY*

EDWIN D KILBOURNE

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IN the wake of an influenza pandemic in which some victims died of infection with influenza virus alone (Louria *et al*, 1959), the position of the present writer that the virus of the pandemic did not differ in virulence from the viruses of the interpandemic disease would appear paradoxical, if not untenable However, this paper will contend that persuasive evidence is lacking that this most mutagenic of human viruses has ever varied in its virulence for man, and that the undeniable occurrence of variation in the severity of influenza, the disease, is usually demonstrably linked to variation in the host or his environment.

It is tautological, but worthy of emphasis, that the "virulence" or severity of infection is a reciprocal of host susceptibility Since in the lexicon of Webster (or Oxford) there is no such word as virulence, in the modern microbiological sense, we are all obliged to essay briefly into semantics—with, we trust, a minimum of

which enable it to induce disease Thus, any virus which produces disease may be termed virulent, and the measure of virulence is the presence or severity of disease It is evident that comparative and quantitative measurement of virulence among virus strains demands a comparison of their effects (disease) in similar hosts under similar conditions Thus, the measurement of virulence is reciprocally related to host susceptibility

Susceptibility in the present context is defined not as vulnerability to the acquisition of infection but rather as susceptibility to those effects of infection which lead to the development of disease By this view, the reciprocal of host susceptibility to the acquisition of infection is the property of viral communicability This capacity of virus to spread from host to host and initiate infection—important as it is—is not necessarily relevant to the outcome of infection in the individual host but influences only the morbidity rate in the total population

Before specific consideration of influenza virus and man it may be useful to consider in general the biological attributes of a virus which might be expected to contribute to its virulence These are

(1) ability to make effective contact with host cells (to infect and survive in host cells)

(2) ability to achieve high concentrations rapidly in host tissues (the resultant of synthesis and destruction of virus),

(3) ability to effect a high ratio of damaging or infective particles to harmless or non infective particles (to preclude the limiting effects of auto interference)

(4) ability to spread within (invade) the host to reach vulnerable sites

(5) ability to impair host function through damage to host cells

(*a*) directly (primarily) or

(*b*) indirectly (secondarily) as the result of inflammatory response to primary cell damage

(6) The ability of a virus to stimulate or pave the way for secondary bacterial infection should be viewed as a sort of pseudovirulence which initiates indirectly a worsening of disease This result is obviously not a real reflection of intrinsic virus virulence, as the worsening of disease will depend upon the nature and vagaries of the bacterial invader

As we review those factors which contribute to the susceptibility of the host to the effects of virus infection we note that they constitute what might be termed the endogenous environment o fection These factors include

(1) lack of prior experience with the virus (absence of immunity),

(2) lack of natural (species) immunity for the v genetic resistance (the latter unproved in man),

(3) impaired ability to reduce the infecting dose of virus by natural inhibitors, pH change, etc at the site of infection,

(4) inability to contain or limit intercellular viral spread or invasion (as by maintenance of normal cell physiology, phagocytosis, ciliary activity, etc),

(5) reduced capacity to resist cell damage and resulting physiological dysfunction

These points will be elaborated further with specific reference to [illegible]

[illegible]

duration of immunity even following known infection and the multiplicity of antigens to which the host may be exposed The final three factors may be influenced by underlying disease, pregnancy or the administration of drugs

In the past, much consideration has been given to the exogenous environment in which epidemics occur—to such factors as weather, season temperature and crowding None of these save crowding is consistently associated with variation in the disease It is probable that the mechanism by which crowding increases both morbidity and mortality rates is not related to the host but to the virus, in that confinement of infection to a limited area must increase the concentration of virus and hence the inoculum or dose of virus to which a subject is exposed This factor of dose is largely [illegible]

[illegible]

virus underlies the success of the primitive variolization methods [illegible]

[illegible]

virulence of influenza virus as observed in man to its biological activity as observed in the laboratory?

To study this question we must make a temporary judgment that influenza viruses *do* differ in virulence for man and may assume, for example, that pandemic viruses or isolates from fatal human disease

differ in virulence from those from patients with mild infection We may then compare these viruses with respect to those properties which they manifest in the laboratory—with specific reference to the hypothetical attributes of virulent virus mentioned earlier

Studies previously reported from this laboratory (Kilbourne, 1959) have indicated that among the pandemic strains of 1957–58 no difference was discerned among strains from fatal or non-fatal infections, even when isolates directly from the lungs of primary, abacterial pneumonia were examined Because definitively, severe or fatal infection with a respiratory virus is dependent upon its capacity to invade and induce dysfunction of the lower respiratory tract, it seems appropriate to examine the "pneumotropicity" of the virus in laboratory mammals The results of many experiments in this laboratory may be summarized by stating that the capacity of first or second egg passage pandemic isolates to induce pneumonia in the mouse did not differ from that of earlier interpandemic viruses, nor was the number of chick embryo infectious doses necessary for murine infection significantly different from other strains not adapted to the mouse The ratio of chick embryo infective/mouse infective virus has been employed as a measure of the mouse virulence of influenza viruses (Kilbourne, unpublished data, Ginsberg 1953)

In Table I are summarized the results of studies of other viral

Table I

THE COMPARATIVE OCCURRENCE OF CERTAIN BIOLOGICAL PROPERTIES IN PANDEMIC (A2) INFLUENZA VIRUSES AND EARLIER INTERPANDEMIC STRAINS

Property	*Possessed by influenza viruses*			
	A2	*A1*	*A*	*B*
Murine pneumotoxicity	+	+	+	+
Murine neurotoxicity	+	+	+	+
In vitro cytopathogenicity	+	+	+	+
Filamentous morphology	+	+	?	+
Sheep RBC agglutination at 30°	+	0	0*	+†
Equivalent amniotic and allantoic sac susceptibility (10–11 day chick embryo)	+	0	?	?
High neuraminidase activity	+	0	0	?

* Laboratory strain NWS agglutinates sheep erythrocytes at this temperature.
† Egg isolated strains post 1950
A A1 A2 is the recently recommended nomenclature of the WHO Expert Committee on Respiratory Virus Diseases for the 3 major antigenic families of influenza A virus

properties in this laboratory and others It is evident that pandemic strains, whether from fatal or non-fatal human infection, did not differ significantly from one another nor from non pandemic strains with respect to cytopathic effect in tissue culture, neurotoxicity in mice (Seto, Hickey and Rasmussen, 1959) or morphology in early chick embryo passage However, the agglutination of sheep erythrocytes by the pandemic viruses at 30°, the ease with which they may be isolated in the allantoic sac (Kilbourne, [illegible] d

cult to correlate such traits with virulence, as egg-line influenza B strains (assumed to be less virulent than A) also possess sheep cell

[illegible]

prompted the examination of pandemic and pre pandemic strains and "fatal" and "non-fatal" isolates with regard to their sensitivity to heat labile and -stable inhibitors in animal sera (Table II) All viruses examined had been passaged only in chick embryos and all but the FM-1 strain had had less than 10 passages In brief, it may be stated that sensitivity to neither heat-labile nor heat stable inhibitor could be correlated with the pandemicity or interpandemicity of a virus, with the year of its isolation, nor with ts site of origin in the respiratory tract nor with its attendance upon mild or fatal disease In fact, the lung isolate from fatal case A/270 (Louria *et al*,

sensitive and inhibitor-resistant, and that graded differences in the inhibitor sensitivity of various strains is explicable on the basis of the relative proportions of the two types of particles In view of [illegible] the apparent [illegible] resistance or

he pristine nature of the isolate Thus, it would appear that we ust turn reluctantly from the laboratory for the definition of fluenza viral virulence and return to a critical study of influenza

Table II

INHIBITION BY ANIMAL SERA OF SEVERAL INTERPANDEMIC AND PANDEMIC INFLUENZA A (EGG PASSAGED) VIRUSES

					Log_2 titre of haemagglutinin inhibitor of 4–8 HA units						
					calf		*guinea pig*		*horse*		
Virus	*Year*	*Place*	*Source*	*Fatal case*	*not Δ*	*Δ*	*not Δ*	*Δ*	*not Δ*	*Δ*	*Resistant to labile inhibitor*
FM 1	1947	N J	throat	0			<1	2	1	1	+
IA 2	1951	N Y	throat	0			<1	1	1	1	+
A/126	1953	La	throat	0			<1	4	1	1	+
A/150	1953	La	throat	0			<1	2			+
A/9	1956	Conn	throat	0	3	3	>3	>3	<1	<1	0
Jap 305*	1957	Japan	throat	0	2	4	6	8	9	8	0
A/201†	1957	N Y	throat	0			<1	<1			+
A/201‡	1957	N Y	throat				2	2			0
A217	1957	N Y	lung	+	<1	<1	<1	2	1	1	+
A217	1957	N Y	throat		<1	<1	<1	3			+
A270	1958	N Y	lung	+	1	<1	<1	6	2	3	0
A270	1958	N Y	throat		<1	<1	<1	<1	<1	1	+
A/486	1959	N Y	throat	0	3	4	7	8	6	7	0
A/493	1959	N Y	throat	0	1	<1	1	4	1	3	+
A/535	1959	N Y	lung	+	1	<1	<1	<1	<1	1	+

Δ 56° for 30 minutes
HA units—haemagglutinating units of virus
* Derived from original inhibitor-resistant strain by limiting dilution passage
† Amniotic isolate—allantoic passage.
‡ Allantoic isolate—allantoic passage

in man for our assessment of influenza virus virulence It may be that the proper study of influenza is man

How shall we judge the virulence of influenza virus for man?

(1) By the severity of *infection* as manifested by the ratio of clinically apparent to inapparent infections (*What proportion of serologically diagnosed infections does not result in disease?*)

(2) By the severity of the (uncomplicated) disease This may be assessed by such relatively objective criteria as the degree and duration of fever and the incidence of signs of bronchiolar or pulmonary disease

(3) By the case fatality rate of the disease

Any interpretation of these aspects of infection in terms of virus virulence *per se* must be done with full cognizance of the exogenous and endogenous (host) environment in which infection occurs, and with recognition of the limitations implicit in a comparison of results obtained under field conditions by different investigators at different times with different methods

Data on the incidence of clinically inapparent serologically diagnosed infections are scanty but are sufficient for a tentative comparison of epidemics associated with all three major antigenic types of influenza A virus (Table III) The variability in the

Table III

PERCENTAGE OF CLINICALLY INAPPARENT INFECTIONS (SEROLOGICALLY DIAGNOSED) WITH VARIOUS STRAINS OF INFLUENZA A VIRUS IN DIFFERENT EPIDEMICS—A MEASURE OF SEVERITY OF INFECTION

Virus	*Epidemic year*	*Population*	*% with antibody rise but no disease*	*References*
A	1943	military camp	57	Commission on Acute Respiratory Disease (1948*a*)
A1	1950	children's institution	32	Kilbourne Anderson and Horsfall (1951)
A2	1957	hospital ward	28	Blumenfeld *et al* (1959)
A2	1957	home	48	Hennessey and Davenport (1959)
A2	1957	high school children	25	Jensen Dunn and Robinson (1958)
A2	1957	mental institution	13	Stallones and Lennette (1959)

Andrews and McDonald (1955) with a similar population in a 1954 epidemic in Warwickshire England and probably reflects the notoriously poor hygiene of such populations Despite the dis similarity of the other populations studied it must be concluded that severity of infection—as measured by the percentage of in fections which induced disease—did not differ strikingly among the three virus types

The severity of uncomplicated disease as a measure of virus virulence

The classical description of influenza as a three day fever is fully confirmed by the data presented in Table IV from a variety of studies of infections with all major antigenic types of influenza A virus The somewhat higher maximum fever noted in two studies of patients infected with pandemic A2 virus may be related to the youth of these two groups or may reflect the activity of a more virulent virus The brevity of the total febrile course would seem to belie the latter interpretation The varying percentage of patients with auscultatory or roentgenographic evidence of lower respiratory tract involvement may well reflect the equivocal objectivity of this clinical parameter Nevertheless the data do not suggest significantly d ffering human pneumotropicity of the three viral types

The clinical course of experimental infection of man

It would seem that study of the course of experimentally induced influenza in man would provide valuable comparative data in a situation in which homogeneity of virus and equivalence of dose may be assured However the relatively few experiments which have been undertaken have utilized viruses of markedly different animal passage history and analysis of clinical data has been less than ideal Thus reported differences in serological and clinical attack rates in these studies are more probably related to experi mental conditions than to differences in the virulence of the original virus prototypes It is of interest that the experiment of Bell and

Table IV

FEVER AND SIGNS OF LOWER RESPIRATORY TRACT INVOLVEMENT AS OBJECTIVE CRITERIA FOR ASSESSING THE SEVERITY OF INFLUENZA ASSOCIATED WITH VARIOUS VIRUS STRAINS

Virus	Epidemic year	Population	Patients		Fever		Pulmonary signs* %	Reference
			No	Age	Ave duration in days	Ave max °F		
A	1936–37	mixed			2 6–4 4	101 2	13 0	Stuart-Harris, Andrewes and Smith (1938)
A	1939	mixed	138		2 3	—	—	Horsfall, Hahr and Rickard (1940)
A	1943	military	79			50% had 102 or more	11 0	Commission on Acute Resp Disease (1948b)
A1	1947	military	252	19	2 4	101 3	3 7	Kilbourne and Loge (1950)
A1	1947	boys' school	57		2 8	101 0		Sigel *et al* (1948)
A1	1949	boys' school						Sigel *et al* (1950)
A2	1957	scout camp	616	13 4	2 0	102 1		
A2†	1957	prisoners	23	21–57	<3 0 in 60%		7 5	Podosin and Felton (1958)
A2	1957	girls' camp			2 in 53%	101–102	5 4	Bell *et al* (1957)
A2	1957	hospital ward	30	18–63	2–4	—	10 0	Blumenfeld *et al* (1959)

* By auscultation or roentgenogram

† Experimental infection with nasal washings.

co workers (1957) with human nasopharyngeal washings induced disease quite comparable to that of natural infection, and that the pandemic (A2) virus employed was similarly associated with some symptomless infections There is an interesting suggestion as one reviews the data on experimental infection that influenza strains isolated and passaged in mammals may retain human virulence in contrast to strains subjected to repeated egg-passage (Isaacs and Roden, 1956)

The contribution of concomitant or underlying disease to the severity of influenza

Although severe and fatal influenza has probably occurred in healthy young adults in the absence of demonstrable bacterial infection, the great majority of carefully studied cases of fatal influenza have been associated with underlying disease or attended by bacterial pneumonia The experience at the New York Hospital-Cornell Medical Center in 1957–58 emphasized the specific importance of cardiopulmonary disease and, still more specifically, rheumatic heart disease with pulmonary hypertension, in the genesis of fatal influenza This experience is summarized in Table V

In the New York Hospital studies the importance of coincident or secondary pulmonary bacterial infection was again recognized, and pneumococci and staphylococci were frequently isolated from severe or fatal cases of influenza However, the bacterial infections posed no important therapeutic problem When death occurred—as it did in more than one third of 30 patients with pulmonary involvement—in but half the fatal cases were bacteria contributory and in these the concomitant rôle of the virus in the production of the diffuse haemorrhagic pneumonia was clear Nine of the 11 who died had serious antecedent disease In a society which nurses to adulthood the congenital and rheumatic cardiac behind a screen of antibacterial agents the relatively increasing threat to life of even the avirulent respiratory viruses is obvious

Not only may pre existing disease dramatically convert a benign infection into a malignant one, but more subtly it may augment the severity of influenza as measured by the reasonably objective criteria of fever or bronchiolitic chest signs In an analysis by Kilbourne and Loge (1950) of the symptomatology induced by the

Table V

THE ASSOCIATION OF UNDERLYING DISEASE WITH PULMONARY COMPLICATIONS OF INFLUENZA
THE IMPORTANCE OF ANTECEDENT CARDIAC DISEASE

(From Blumenfeld *et al*, 1959 Reproduced by permission of the Editors, *J clin Invest*)

Disease syndrome	*No of patients*	*Incidence of underlying disease**			*Deaths*		
		Total no patients	*No with CV disease*	*No with RHD*	*Total no who died*	*No with underlying disease*	*No with RHD who died*
Influenza complicated by bacterial pneumonia	15	8†	3	3	1	1	1/3
Concomitant influenza virus and bacterial pneumonia	9	7†	5	3	5	3	1/3
Primary influenza virus pneumonia	6	6	6	4	5	5	3/4
Totals	30	21	14	10	11	9	5/10

* CV, cardiovascular disease; RHD rheumatic heart disease
† In one of this number the "disease" was pregnancy

1947 virus, it was noted that those patients in whom routine throat cultures were positive for B haemolytic streptococci experienced higher maximum temperature elevation and more than twice the frequency of chest signs as those in whom streptococci were not detected

A study by Podosin and Felton (1958) of Asian influenza in 616 Boy Scouts disclosed that 27 per cent of those patients with a history of asthma had abnormal chest roentgenograms in comparison with the 7 5 per cent abnormalities found in patients without

Even the pattern of symptoms may be influenced by underlying disease or the administration of drugs In the New York Hospital studies (Blumenfeld *et al*, 1959) an unwarranted incidence of gastrointestinal symptoms in previously bed ridden patients was attributable to the administration of drugs potentially disturbing to gastrointestinal function

It is significant that in earlier reports of fatal influenza in interpandemic periods the probable contribution of underlying disease is apparent, if not always emphasized In 7 of 20 cases proved by pulmonary virus isolation prior to 1957, 6 had coexisting disease, in 8 cases in which no clinical details were furnished all but one had evidence by culture of staphylococcal or pneumococcal pneumonia the 4 cases clearly without antecedent disease had bacterial pneumonia Thus in the purview of modern virology it may be said that not only is the fatal case of influenza rare, but it is the rare case in which death occurs in the absence of antecedent disease or coincident bacterial infection Death associated with influenza virus as the sole pathogenic micro organism is almost invariably restricted to those with antecedent cardiopulmonary disease

On the other hand, the inroads of demonstrably virulent virus in high dosage may be sharply contained by the host which has experienced prior infection This point is epitomized in a single experiment which is summarized in Table VI In this experiment, mice of the same strain, age and sex were first infected with an avirulent (non mouse adapted) strain of FM-1 virus (A1 1947) or injected intranasally with control material Thirteen days after

Table VI

DIFFERING EFFECTS OF IDENTICAL INOCULA OF VIRULENT INFLUENZA VIRUS IN COMPARABLE HOSTS (MICE) WITH VARIED IMMUNOLOGICAL AND PHYSIOLOGICAL EXPERIENCE

	Experience		*Severity of disease induced by virulent challenge virus**		
Mouse group	*Previous infection with avirulent FM-1 virus*	*Injected with cortisone*	*D/T†*	*Lesion score‡*	*Virus in lung§*
1	0	0	4/6	72	
2	0	5 mg	4/5	80	
3	+	0	0/6	0	0
4	+	5 mg	0/6	10	+
5	+	5 mg × 3	0/6	33	+

[illegible] pension infects

this initial experience (which all survived) certain of both control and infected mice were injected with cortisone in moderate or high dosage prior to challenge with a virulent FM-1 strain of virus The severity of the disease in these mice of differing immunological or physiological experience was assessed on the basis of mortality rate, the degree of grossly manifest pulmonary consolidation and the persistence of virus in the lungs of survivors This experiment clearly shows not only the expected result that prior antigenic experience dramatically negates the severity of infection induced by intrinsically virulent virus, but that even this high order of resistance may be broken down by changes in the physiological experience (endogenous environment) of the host, as in this instance by administration of cortisone just prior to reinfection It is notable that the maximal dose of cortisone employed in this experiment is inadequate to suppress antibody formation to influenza virus in mice (Kilbourne, 1955)

1918-19 revisited

Now that a modern [illegible] has [illegible] its aetiol[illegible] to a [illegible] se of [illegible] virul [illegible] he i[illegible] more

1933, how does it compare with the scourge of 1918–19? Although the total morbidity of the World War I outbreak probably did not exceed that of the recent pandemic, the total mortality certainly did, and high case fatality rates in young adults contrasted with usual experience in other epidemics (including 1957–58) of mortality peaks only at both ends of the age spectrum The writing of still another agonizing reappraisal of the 1918 experience must be defended, as the virus of the outbreak (unless it be the swine virus) is still not at hand But as it is the contention of this paper that study of the host and his environment are more crucial to the interpretation of virulence than laboratory study of the virus itself, it may be of more than academic importance to ask whether the differences in our last two pandemics are explicable without invoking the bogey of changing virus virulence

At the outset of this discussion it should be recapitulated that the best documented fact of 1918–19 is that all but a few who died of "influenza" died with, if not of, bacterial pneumonia (MacCallum, 1919, Wolbach, 1919, Opie *et al* 1921) The aetiological heterogeneity of these pneumonias was both recognized and emphasized at a time when Pfeiffer's bacillus was the much disputed contender for the rôle of aetiological agent of influenza itself To be explained, then, is the undue prevalence of bacterial pneumonia in young adults and especially young men First, one asks if uncomplicated influenza (as a predecessor to the bacterial sequel) was more common in this age group It was not Rather the case fatality rate of those in the 20–40 age group was excessive (Jordan 1927) If we then infer that this age group suffered more secondary bacterial pneumonia, we must ask, why so?

As the host's endogenous environment contributed to the fatalities of 1957–58 then the exogenous environment of the 1918 training camp was similarly important in the earlier pandemic If it was the worst of times for the poor recruit, it was the best of times for the student of bacterial infection Meningococcal pneumonia and pericarditis, *Hemophilus influenzae* pneumonia, streptococcal empyema—such bizarre manifestations of infection monopolize the journals of the period as chronic diseases do today Are we to say that the virus of measles was also more virulent at this time because 181 of 2,956 cases of measles at Camp Riley Kansas, contracted "measles pneumonia" (streptococcal) and 83 died (Stone, Phillips and Bliss, 1918)? (A pneumonia case fatality rate of 45 8

per cent) Pneumonic complications of measles were unusual in overseas (AEF) troops at a time when 4 5 per cent of those with measles in training camps developed pneumonia (Jordan, 1927)

It may well be questioned whether such bacterial devastation would have followed the recent pandemic even in the absence of

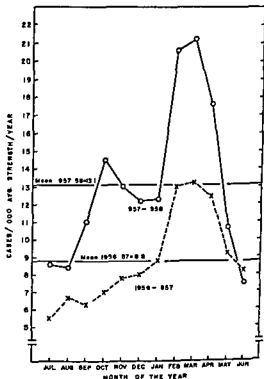


FIG 1 Comparative incidence of pneumonia (all forms) active duty U S Army personnel (world wide) during 1956–57 (pre pandemic) and 1957 58 (pandemic) year

antimicrobial drugs A partial answer is provided by the data in Fig 1 which details the differing pneumonia incidence in the U S Army in the year preceding and the year of the 1957–58 pandemic If a pneumonia case fatality rate of 25 per cent is projected from these data [in consideration of age, sex, military status and probable

pneumococcal aetiology (Heffron, 1939)] the potential virulence of the recent pandemic may be partially assayed On the basis of mean increase of pneumonia incidence in the influenza year of 4·3 cases/1000 average troop strength, an increase in mortality of 25 per cent of 4 3, or 1 07 deaths/1000 would have occurred in the presulphonamide antibiotic era The total strength of the army was 932,162 in this period, so that approximately 1000 lives might have been lost in earlier times But we can never measure nor even guess at the reduction in secondary cases of bacterial infection effected by prompt antimicrobial treatment of the initial ones

Finally, let us examine the severity of the *uncomplicated* disease of 1918–19 as an index of the virulence of its virus To begin with, most contemporary students of the disease (Jordan, 1927, Lichty, 1919, Bloomfeld and Harrop 1919) recognized a brief, benign, uncomplicated illness distinct from the more protracted disease with pneumonic involvement Although clinical descriptions of the time were excellent, and even literary it is difficult to find flat quantitative statements concerning fever duration and the incidence of minor chest signs Even Lichty's fine study is confusing on this point Having stated that the average duration of fever in soldiers without "inflammation of the lungs" was 6½ days, he later states that "when the temperature remained up longer than *five* days, it could safely be concluded that lung involvement must be present" Still later in the same report the writer implies a mean fever duration of 3–4 days In his exhaustive review, Jordan (1927) states that 'the fever usually lasts 2–3 days in the simple 'uncomplicated' cases'

The finding of Bloomfeld and Harrop (1919) that only 6 per cent of 300 patients had pulmonary rales (but no roentgenographic evidence of infiltrates) does not suggest any unusual pneumotropi city of the 1918 virus and supports the statement that ' Roentgen rays (did) not afford a means of diagnosing the uncomplicated influenza" (Jordan, 1927)

If one accepts the importance of bacterial complications in the fatal outcome of influenza then the greater "virulence" second wave in the winter of 1919 may be to the the year in which it occurred—a t respiratory bacterial pathogens and

It is suggested that the d resulted from the fortuitous

[illegible]

dissemination and high dosage of virus but spread of bacterial pathogens to an unusual degree

A note on reduced virus virulence between epidemics

The *foregoing remarks on virus virulence as a reciprocal of host* susceptibility may be extended in explanation of the "disappearance" of influenza virus between influenza virus outbreaks and the postpandemic waning of disease severity As the occurrence and severity of clinically manifest disease is influenced by preexisting antibody levels, there seems no reason to doubt the probability that influenza virus infection continues in sporadic, mild and unrecognized guise as population antibody levels rise Influenza virus infection in the absence of disease has long been recognized (Table III), and it is now established that virus may be recovered from the inapparently infected (Bell *et al*, 1957) The unaltered potential of the interepidemic virus to cause severe disease is suggested by the occurrence of fatal cases of bacterial [illegible] presence in the lungs of patients [illegible] faxwell, [illegible]

In *summary, it is* concluded that the host parasite relationship of man and influenza virus is a tenuous one subject to wide variation in the incidence and manifest severity of the resultant disease On the evidence that morbidity is conditioned by the presence or absence of immunological experience and mortality by abnormalities of the host or his environment, it is *concluded that variation in* the intrinsic virulence of influenza virus for man has not demonstrably *occurred and need not* be postulated to explain variation in the severity of the disease

DISCUSSION

Morgan In regard to the data that you presented, Dr Kilbourne, on the ratio of clinical cases to individuals who show antibody rises, is it possible to analyse the situations and determine the degree of

saying this, one feels that probably there is just as much subclinical immunization in the latter situation. Then there is the problem of comparing different ages, because whereas the Asian influenza

these two variations of the infection It may be that influenza is

composition

Kilbourne I fully agree Furthermore, one could, I think, explain the persistence of influenza virus interepidemically by assuming that such mild infections would occur in populations with high antibody levels without postulating any animal vectors or intermediate hosts as reservoirs of infection between epidemics

I was impressed with the paucity and inadequacy of the data which I tried to examine, but I have done so with the conviction that

THE VIRULENCE FOR MAN OF SOME RESPIRATORY VIRUSES PASSED IN TISSUE CULTURES

D A. J TYRRELL AND Γ E BUCKLAND

M R C Common Cold Research Unit Salisbury

THE facilities of the Common Cold Research Unit have been used in the past mainly for studies on experimental epidemiology attempts to cultivate the virus, and other aspects of the common cold (Andrewes 1958) In the past two years we have added to this work by testing the effects in volunteers of a variety of viruses which have been recovered from the human respiratory tract The viruses used were chosen because they were thought to be unlikely to produce more than a minor respiratory illness In some cases we wished to know whether the virus could produce symptoms like *those called a common cold In other cases we hoped no symptoms* would be produced but that there would be an antibody response

variety of results, and they are presented here in such a way as to illustrate the types of clinical and laboratory changes observed These are all related we believe to the larger matter of the general concept and measurement of the virulence of viruses for man However these experiments were primarily designed to show whether certain viruses could cause colds and whether others had possibilities as live vaccines They were terminated when these questions were answered Although the data might have been added to in order to answer more academic questions on virulence this was not thought justifiable

We isolated the volunteers and assessed their illnesses by methods previously developed at this unit (Andrewes 1948, Roden 1958) The observer who examined the volunteers and made the diagnosis was unaware whether volunteers had received virus or control material and usually did not know what virus was being used The laboratory methods for the experiments with

influenza were those in general use The methods employed with other viruses were generally similar to those used by previous workers with the viruses concerned All volunteers were isolated for 2 or 3 days and then received the virus in 1 ml of balanced salt solution as nose drops Observation was continued for a further 7 or 6 days and specimens for virus isolation were collected on alternate days Paired sera were obtained from almost all volunteers. Antibodies were measured by several techniques but for the purposes of this discussion antibody will mean neutralizing activity detected using about 100 TCD_{50} of virus In the case of influenza virus the only data available are haemagglutination inhibition titres of cholera filtrate treated sera The results of these trials will be reported in full elsewhere

ECHO viruses

As their full name suggests (enteric cytopathogenic human orphan viruses) these viruses are most readily found in the faeces of normal children (Committee on Enteroviruses 1957) but we have studied two which were found in the respiratory tract and faeces of children with acute febrile illnesses with marked respiratory symptoms (Fig 1)

A strain of ECHO 11 (U virus of Philipson and Wesslén 1958) was given in a dose of 10^5 tissue culture infectious doses (TCD_{50}) The results are summarized in Table I All volunteers who received the virus became infected even though 4 of them had low levels of serum antibody before inoculation Although virus was

Table I

EFFECT OF INOCULATING HUMAN VOLUNTEERS WITH ECHO TYPE 11 VIRUS (U STRAIN)

Tissue culture passages of strain given	*Dose* TCD_{50}	*Virus isolated*	*Antibody rises*	*Illness produced*†
3 HEL*	100 000	9/9	9/9	8/9
3 HEL +11 MK	100 000	8/8	7/8	0 8

* In this and following tables, HEL = human embryo lung HEK = human embryo kidney and MK = monkey kidney

† In this and following tables all the illnesses which were observed occurred in volunteers from whom virus was recovered or showed an antibody rise or both.

recovered from the throat in almost all cases there were practically no respiratory symptoms Those volunteers who became ill suffered from a general illness with headache, malaise and fever accompanied by disturbances of the alimentary tract—abdominal distension and discomfort and diarrhoea The only significant illnesses were found in those volunteers receiving virus passed 3 times in human embryo lung cultures Virus passed 11 times further in monkey kidney cultures produced no definite symptoms This may be related to the fact that it apparently multiplied less well in man than third pass virus, certainly virus isolations from the throat were less frequent and the mean titres of virus in throat swabs and in faeces were about tenfold less Nevertheless after infections with both kinds of virus we found a rise in serum antibody levels in all volunteers and the mean rise was about 100 fold in both groups

ECHO 20 (Cramblett *et al*, 1958) was tested in the same general way and some of the results are summarized in Table II

Table II

EFFECT OF ECHO TYPE 20 VIRUS

Tissue culture passage of strain given	*Dose TCD_{50}*	*Virus isolated*	*Antibody rise*	*Illness produced*
0	5	2/4	0/4	0/4
0	20–30	3/4	2/4	1/4
0	300	4/4	2/2	3/4
3 HEK	100 000	3/3	0/0	1/3
3 HEL	100 000	3/3	0/0	3/3

In this case we were able to use the virus contained in swabs taken from sick children and sent to us from Bethesda by Dr L Rosen We also gave larger amounts of the virus after passage in tissue

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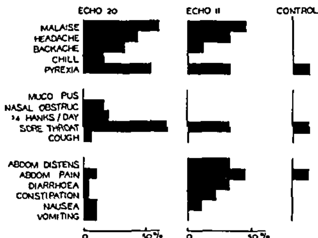


FIG 1

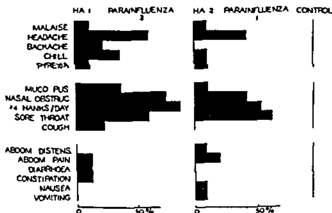


FIG 2

FIGS 1 and 2 The frequency of virus symptoms and signs in infected with virus >4 Hanks/Day means that the volunteers more than four paper handkerchiefs in one day, i e that there significant increase in nasal secretion

produced resembled those found with virus from throat swabs There was one case of coryza and three of general influenza l ke symptoms

Parainfluenza viruses

The name parainfluenza has recently been coined to tidy up the classification of some recently isolated respiratory viruses (Andrewes *et al* 1959) Sendai and haemadsorption virus Type 2 are grouped together as strains of parainfluenza virus Type 1 Croup associated and ALTB viruses become parainfluenza virus [illegible]

al 1959)

We used strains of HA1 and HA2 viruses which had been isolated recently in Britain (Sutton Clarke and Tyrrell 1959) and in Denmark (Petersen and von Magnus 1958) The results ob tained are summarized in Tables III and IV and Fig 2

Table III

EFFECT OF PARAINFLUENZA TYPE 1 VIRUS (COPENHAGEN 222 STRAIN OF HA2)

Tissue culture passages of strain given	*Dose TCD_{50}*	*Virus isolated*	*Antibody rise*	*Illness produced*
1 MK	15	4/5	3/5	3/5
2 MK	1 5	2/2	0/2	1/2
5 MK	15	0/6	0/6	0 6
5 MK	150	3/5	0/5	2/5

Viruses of several passage levels were used and the dose given varied widely Only volunteers who were infected became ill The illnesses observed after inoculation of parainfluenza Type 1 were characterized by coryza sore throat and slight malaise without fever or abdominal symptoms These results are generally similar to those of Reichelderfer and co workers (1958) There was no obvious clinical difference between the illnesses produced by large and by small doses of virus of any number of passages in tissue culture However antibody responses were detected in infections

Table IV

EFFECT OF PARAINFLUENZA TYPE 3 VIRUS (MOSS AND K STRAINS OF HA1)

Tissue culture passages of strain given	Dose TCD_{50}	Virus isolated	Antibody rises	Illness produced
K 2 MK	15	0/3	0/3	0/3
K 2 MK	1 500	2/2	1/1	2/2
Moss 5 MK	15	0/3	0/2	0/3
Moss 5 MK	1 500	1/4	1/4	1/4
Moss 5 MK	15 000	1/4	1/3*	1/4
Moss 5 MK	150 000	3/4	3/4	3/4

* Not the volunteer from whom virus was isolated and who was ill.

with the first pass of the parainfluenza Type 1 virus and not with the second or fifth pass

In general the clinical symptoms associated with Type 3 were identical with those produced by Type 1 infections and by a pedigree common cold. However a sharp attack of clinical influenza was produced in one volunteer by 1 500 TCD_{50} of second pass K virus In addition virus from the volunteers receiving Moss virus was relatively cytopathic for monkey kidney cultures while that recovered from volunteers who were given K virus was non-cytopathic For ethical reasons the experiments with the virulent strain were not extended All volunteers receiving parainfluenza Type 3 virus had detectable circulating antibody at the time of inoculation Antibody responses occurred in infections with both strains of parainfluenza Type 3 virus

Comparative titrations of viruses in man and in tissue cultures might be a useful way of studying virulence They are clearly impracticable but it can be seen in Table III that the infectious dose for man of the fifth passage virus of parainfluenza 1 apparently lies between 15 and 150 TCD_{50} while the second passage virus is infectious when only 1 5 TCD_{50} is given We expected the ratio of the infectivity titres of these viruses in man and in tissue cultures to be correlated with the relative virulence for tissue culture and for man and these results certainly suggest that the adaptation of the virus to man was falling as the virus was passed in tissue culture However the results of similar inoculations of parainfluenza Type 3 strains in man show that a correlation like this may not apply when different strains are compared

Adenoviruses

It has been shown that infection of human volunteers with [illegible]

passed several times in monkey kidney cells (Heubner *et al* 1955) In an attempt to produce an asymptomatic infection our col [illegible]

antibodies when inoculated Most of those inoculated were infected and marked rises in neutralizing antibody occurred though there was no increase in complement fixing antibody The results are summarized in Table V Even 150 TCD_{50} of virus

Table V

EFFECT OF ADENOVIRUS TYPE 7

Tissue culture passages of strain given	*Dose TCD_{50}*	*Virus isolated*	*Antibody rises*	*Illness produced*
17 MK+7 PK	15	0/2	0/2	0/2
17 MK+7 PK	150	1/2	1/2	0/2
17 MK+7 PK	1 500	1/2	1/2	0/2
17 MK+17 PK	150	0/2	1/2	0/2
17 MK+17 PK	15 000	2/3	2/2	0/3

passed 7 or 17 times in pig kidney cultures produced infection in volunteers This represents a 1/3000 dilution of culture material Fever up to 99 8° occurred in 1 volunteer but no symptoms amounting to a definite illness were noted All the volunteers who resisted infection had detectable serum antibody when inoculated except for the one receiving 150 TCD_{50} of 17th pig kidney passage virus An attempt was made to detect changes in the virulence of viruses obtained from volunteers These viruses retained their pathogenicity for pig kidney cells and when virus contained in throat swab material (about 10 TCD_{50}) was passed to 5 further volunteers only 2 became infected No illnesses characteristic of adenovirus infections were produced However, one infected and one uninfected volunteer developed the symptoms of a cold and this may well represent the activation of an ordinary cold virus

the appearance of a common cold virus during an attempted serial passage of adenovirus has been observed at this unit (Pereira and Roden, unpublished data)

Influenza viruses

Russian workers have extensive experience of preparing attenuated strains of influenza virus for administration to man (see Zhdanov, 1959) Earlier work at this unit suggested that, during adaptation to eggs strains of influenza virus tended to pass rapidly from a state in which they could infect man and cause an attack of mild influenza to a state in which they failed to infect at all (Isaacs and Roden, 1956, Isaacs, Negroni and Tyrrell, 1957) In collaboration with Dr L Fadeeva we inoculated volunteers with two Russian vaccine strains The results of the first experiments are shown in Table VI. No clinical symptoms at all were noted but

Table VI

EFFECT OF INFLUENZA VIRUS A 1955 RUSSIAN VACCINE STRAIN

Passages of strain given	*Dose EID_{50}*	*Virus isolated*	*Antibody rises*	*Illness produced*
? Egg 7 HEL	1 000	5/5	0/5	0/5
7 HEL	1 000 000	5/5	0/5	0/5
? Egg 7 HEL 1 egg	1 000	1/6	1/4*	0/6

* The antibody rise was detected in the volunteer from whom virus was isolated.
EID = egg infectious dose

virus was recovered from all the volunteers receiving the original vaccine, although most volunteers possessed detectable serum antibody In spite of this there were no rises in serum antibody levels after the infections were detected Sera were titrated at Salisbury and at the National Institute for Medical Research, Mill Hill, and 7 different strains of influenza A virus were used as antigens in haemagglutination inhibition tests [ranging from WS to A (Asian)] Complement fixation tests using crude S antigens were negative This vaccine virus was passed once in eggs at limiting dilutions, and the egg fluid was given to volunteers in doses similar to the original vaccine Only 1 out of 6 volunteers was infected, a

result which suggests that the virus had a decreased ability to initiate infection in man Five of the volunteers had initial serum HI antibody titres of 10 or less against the vaccine virus but others were infected in spite of a serum antibody titre of 80

Discussion

In a straightforward acute infection the virus invades body cells More virus is produced in them and is shed to infect other cells The infected cells are damaged and so illness is produced and the shed virus stimulates the production of antibody If antibody is present when the virus reaches the body then infection does not begin or does not spread because virus particles are neutralized before they reach the susceptible cells

Many of our experimental infections do not fit into this simple picture and we are forced to ask far more questions than we have answered If we assume that a virus adapted to tissue culture can multiply less well in human body cells—as seems to be true with ECHO 11—why did we not observe a relatively smaller antibody response when volunteers were infected with the tissue culture passed virus which produced no symptoms? How can one have enough cells infected by a virus to produce an illness and yet have no antibody response as was apparently the case in some para influenza Type 1 infections?

Perhaps the protein of adenovirus or of an ECHO 11 virus is a better antigen for man than the components of influenza and para influenza viruses Perhaps ECHO and adenoviruses infected or were absorbed from the gut and so gave an antigenic stimulus while in limited influenza and parainfluenza virus infections the virus was shed into the lumen of the nose and swallowed and destroyed in the stomach Whatever may be the correct answer we should recognize clearly that it is possible to have a limited infection with a respiratory virus which may cause enough symptoms to be called a common cold but which may be initiated in spite of the presence of antibody in the serum and may end without producing any increase in circulating antibodies Infections of this sort may often occur in the colds which afflict adults in Nature on the other hand they may never occur in Nature but only in volunteers given rather large amounts of virus in an unnatural manner

Why did we need more tissue culture doses of parainfluenza

Type 3 than of parainfluenza Type 1 to initiate infection in man? It may be a reflection of the speed with which they multiply in and adapt to tissue cultures of monkey kidney cells, or a reflection of the higher titre of serum antibody against parainfluenza Type 1 in our volunteers On the other hand, if we used some other route of infection we might have found that a much smaller dose of parainfluenza Type 3 would initiate infection, and this possibility will be explored in future experiments

In general it seems to us that where, in our experiments, we have seen apparent attenuation of the virus for man this has been the consequence of a limitation in the capacity of the viruses to multiply in man This may have been due either to the host being relatively resistant or immune, or to the virus having lost some of its former ability to multiply in human cells Our experiments suggest that the reduced amount of multiplication leads to a reduction in the intensity of symptoms produced without any qualitative change in the symptoms themselves However, this may not always be so

The type of clinical illness produced by ECHO 20 virus was not apparently modified by passage of the virus in tissue culture of human cells On the other hand, the ECHO 11 virus was first isolated from children with respiratory disease and yet we did not see any respiratory symptoms in the volunteers given this virus Does this mean that ECHO 11 virus was modified by passage in tissue cultures, were the children's illnesses produced by another virus which was not isolated, or is there a difference in the type of symptoms which ECHO 11 produces in children in Sweden and in adults in England? Unfortunately, specimens from patients infected with this virus were not available and these possibilities could not be tested experimentally

Finally, although all the viruses were inoculated in the same way the type of symptoms produced varied with the biological type of the virus introduced It seems therefore, that strictly con trolled volunteer trials can tell us at least something about the varied pathogenic potentiality of viruses recovered from the respiratory tract of man.

Acknowledgements

These experiments would have been impossible without the willing and conscientious help of the volunteers and the staff of the Unit We wish in particular to thank Miss G Worthington for permission to refer to her work on adenoviruses, Dr M L Bynoe and

viruses

DISCUSSION

Dick What tests are used for purity, to exclude from your tissue culture material known pathogens or agents which we do not yet

response to the test virus is due to an infection with a contaminating agent

Tyrrell In general, we obtain a new virus from some other laboratory and cultivate it by the methods used in that laboratory. We check that its general properties are as they should be We check its identity by a neutralization test with specific immune sera, we keep the cultures going for about three weeks to give any contaminating viruses a chance to grow up We have detected contaminating viruses on several occasions mixed up with a new virus, on these occasions we did not inoculate the original virus to volunteers Another safety test is to give the new virus to ourselves We have not

thawed and we had the impression that we were losing a ... in the process of doing that, but odd specimens had titres of up to 10^2 or 10^3 infectious doses per ml

nearly as much virus

id to these positive

irlane Burnet's own



Miss J. Macdonald for help in the clinical work, Dr. C. H. Andrewes

viruses

DISCUSSION

Dick What tests are used for purity, to exclude from your tissue culture material known pathogens or agents which we do not yet know how to isolate? I am reminded of the fact that many people used HeLa cells for a long time, without realizing that many strains are contaminated with PPLO up to 10^8 per ml. One wonders whether in the volunteers given the pig kidney tissue cultures, the symptoms might not be due to ECHO viruses and that no antibody response to the test virus is due to an infection with a contaminating agent

Tyrrell In general, we obtain a new virus from some other laboratory and cultivate it by the methods used in that laboratory. We check that its general properties are as they should be. We check its identity by a neutralization test with specific immune sera; we keep the cultures going for about three weeks to give any contaminating viruses a chance to grow up. We have detected contaminating viruses on several occasions mixed up with a new virus; on these occasions we did not inoculate the original virus to volunteers

thawed and we had the impression that we were losing a lot of virus in the process of doing that, but odd specimens had titres of up to 10^2 or 10^3 infectious doses per ml

Kilbourne How many did you put in?

Tyrell We put in 10^3, so we were getting out nearly as much virus as we put in, some days after we had put it in

Stuart-Harris Thinking of the background to these positive results with influenza, comparing Sir Macfarlane Burnet's own

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Mulder With the egg-lines of Asian virus I got no antigenic response in humans, but as soon as the strain was passed in mice it gave an antibody response I thought from general experience, and also from the experiments of Francis, that the mouse-adapted lines could give responses and protection in volunteers much better than egg lines

Miss J Macdonald for help in the clinical work, Dr C H Andrewes

viruses

DISCUSSION

Dick What tests are used for purity, to exclude from your tissue culture material known pathogens or agents which we do not yet

agent

Tyrrell In general, we obtain a new virus from some other laboratory and cultivate it by the methods used in that laboratory We check that its general properties are as they should be We check its identity by a neutralization test with specific immune sera we keep the cultures going for about three weeks to give any contaminating viruses a chance to grow up We have detected contaminating viruses on several occasions mixed up with a new virus, on these occasions we did not inoculate the original virus to volunteers Another safety test is to give the new virus to ourselves We have not

in serum-rich media

My question is related to Prof Dick's In connexion

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Tyrrell We then took some of that virus and passed it ten times in monkey kidney cells, and it remained in the D phase It produced a cold-like illness in one of the three people infected, but we did not work it out properly

Pereira I understood that the antibody titres in these two experiments were determined by haemagglutination inhibition and complement fixation tests Would it not be possible that you might have had some serum neutralizing antibody rises?

Tyrrell Yes, I suppose it would have been We have heard that certain workers in Czechoslovakia have done experiments like this with Russian A2 strains, and got results almost identical with ours

Burnet In the earliest work in this field, for which we were responsible, in 1940–43 the strain LEE B adapted to egg by Francis and given a good few passages in our laboratory, when given to volunteers produced with fair consistency a rise in antibody in those who had low, basic antibody The A results were not nearly as we got a fair number of rises, enough to test it out on 10 afterwards

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Tyrrell Dr Isaacs, of course, is very critical of these experiments from this point of view We used amniotic or allantoic inoculation to isolate the virus We took throat swabs, or throat swabs plus garglings and nasal washings, at daily intervals from 1 to 5 days

thawed and we had the impression that we were losing a lot of virus in the process of doing that, but odd specimens had titres of up to 10^2 or 10^3 infectious doses per ml

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Kilbourne I have just been reviewing some of the experiments [illegible]

mammals

Stuart-Harris Was the early American work not done with very large doses of virus?

[illegible]

strains that you have recovered at an interval after infection where it is reasonable to suppose that multiplication has taken place, have you put that into volunteers either with or without further tissue culture passage?

Tyrrell Yes We did such an experiment in connexion with the adenovirus work because we were thinking of a live virus vaccine, [illegible]

Kilbourne Dr Tyrrell, you raised another paradox that has to be explained In the case of parainfluenza 1 and the production of [illegible]

human, it may be producing largely non infectious virus, so it is

quantitatively not enough to be an antigenic stimulus, but it is producing *in vivo* cytopathogenic effects which are sufficient to

work to find this out

Buckland We have worked with monkey kidney, and so have

Andrewes In that connexion, the apparent attenuation of a virus which Tyrrell described has, of course, been achieved by passage in monkey kidney, and at the moment we are attempting to get a similar attenuation by passage in human kidney tissue

Stuart-Harris In regard to the question of nasal symptoms in relation to human infection, arguments have been put forward that they are based on an allergic reaction As the parainfluenza viruses are probably agents which circulate widely in the population, I wonder whether symptoms due to them may be primarily due not to

Burnet An interesting possibility there, by analogy with influenza, is that you might have an infection which liberated a great deal of incomplete non infective virus which nevertheless had the allergin This might make it extraordinarily difficult to demonstrate a virus

Andrewes But, if the parainfluenza viruses were doing that, we should be able to pick them up in tissue culture

Morgan What about the effect of antihistaminics?

Burnet Has it been tested?

Morgan I don't know whether it has been tested in this disease It has certainly been tested in respiratory infections that are not defined and I do not think any of the tests offer much hope that this was the mechanism

GENERAL DISCUSSION

Burnet The important discussions of this meeting have centred around two topics, first the evidence for and against variation in [illegible]

variation in influenza virus virulence over the years

Andrewes I am interested in Dr Kilbourne's view that the 1918 influenza virus was not particularly virulent and that virulence of associated bacteria can explain the facts of that pandemic I confess I don't see how that theory explains the curious age incidence of [illegible]

influenza virus, but these bacteria also, got up a certain momentum and spread together not only amongst the soldiery in the war area but also when they got into other countries such as India This is in a way a cheering point of view, because it means that we magnificent

pathogens than on the basis of a change [illegible]

example, and a few others, almost always a bacterial pneumonic [illegible]

Tyrrell Is it not possible, Dr. Kilbourne, that we gain the [illegible]

may indeed produce variations but we are just not good enough at

reached ecologically in man over the years with this virus, and why there should be any particular survival value in a virus being highly virulent

Andrewes· It would be odd that influenza virus should be so very stable as regards its virulence when it is the most labile virus we know as regards its antigenic properties

Kilbourne Except that linkage between antigenic variation and virulence is certainly not demonstrable.

Tyrrell: It seems to me that the mechanism which Dr Andrewes suggested could be operating here The virus is basically variable but is generally kept at such a level of virulence that enough sputum is sprayed around during an infection for other hosts to be affected

[illegible]

lines thus far tested

Pereira Have you tried it in human embryonic cells?

Kilbourne No

Tyrrell Another of our safety precautions is that we never give viruses which have been through any sort of transformed cell lines

Isaacs You would agree, Dr Kilbourne, that Asian viruses can vary in virulence in mice, they can be adapted to mice

Kilbourne Yes

Isaacs I would take the stand that the Asian viruses could also vary in virulence for man Some of the components of virus virulence which have been mentioned today can, perhaps, be brought together One point that was mentioned was the rate of virus multiplication There is quite good evidence in the literature that in some strains, at any rate, the more rapidly multiplying strains are more virulent Antigenic novelty has also been mentioned, and a strain [illegible]

earlier His experiment gave the surprising result that a large dose [illegible]

are measuring when we talk about virulence is simply the ability to reach a critical virus concentration. That would fit in very nicely

Andrewes Just as soon as the level of the swine A antibody in the human population has gone down low enough

Mulder I think that there is a chance that it will go down

Burnet Why has it not escaped into children and young adults already?

Mulder Some children might show swine antibody At the age of 20 years the incidence is about 30–40 per cent, and at the age of 40 it is nearly 100 per cent If a child were to get swine A influenza it would be difficult for the virus to find its way through the population

Morgan There is a great deal of influenza isolation in Iowa

Kilbourne Dr Tyrrell, I recall that you found that a monkey-kidney adapted strain was pathogenic, but pig-kidney adapted

only a few tissue culture doses of parainfluenza virus passed once or

Smith The question of virulence is closely tied up with the

other species If it could be shown that adaptation of, say, egg virus to the mouse is associated with a change of the host component of the virus, a start would have been made *in the determination of a virus* virulence factor

Kilbourne Is there any evidence that, once influenza virus or any *other virus is adapted to an alien host, to a certain state of virulence,* it will *change with passage in reasonably high dilution?* Sir Macfarlane Burnet raised the point of the probability of a very small inoculum in the natural human disease and reminded us of his own experiments concerning the fact that the changeability of virus and the favouring of genetic change is greater with passage at low

moderate virulence, but when introduced into South Africa had a highly lethal effect on most of the antelopes there There is always doubt about what a virus will do when it is transferred from its natural host to an alien host It is very difficult to generalize or to look for specific biochemical reasons for its behaviour

Andrewes Prof Mulder has some justification for his fears about the American reservoir, because if we take the swine A influenza at all seriously in relation to human influenza, we have to believe that it got from man to pig If it jumped one way it might jump the other

tropic for mice proves almost immediately to be pneumotropic for the ferret too and *vice versa* There is the fact that Shope's laboratory swine line is immediately pneumotropic in mice and ferrets I feel [illegible]

[illegible]

vaccines is that they spread, the second is that excreted vaccine viruses cannot be differentiated from some of the less virulent naturally occurring strains Both of these points are generally agreed upon

[illegible]

community However, we cannot prove that excreted virus has com pletely different characteristics from naturally occurring avirulent

imported virus, or was it a change in a naturally occurring avirulent strain which for some reason suddenly became epidemic?

Isaacs Have you any information about the Russian results?

Dick The Russian passage experiments were done in a very artificial way The Russians took faecal vaccine virus and then put it

is that in virulence tests I understand that in some tests monkeys

wrote about this But is is quite unsound to draw a comparison between 17D and vaccinia, because the whole point is that 17D does not spread

Andrewes Ordinary yellow fever spreads and a modified variety called 17D does not Why can you not get a similar situation in polio?

Tyrrell The adenovirus vaccine might be put in this group because the titres of virus in the throat are very low, it is quite difficult to reisolate the virus

Burnet I gather that what you really want is a marker by which you

Dick Exactly

Stuart-Harris Prof Dick argues very strongly that one wants attenuated viruses which do not spread, and yet others are just as vehement that such a vaccine would be almost useless The very situation where you want an attenuated virus is in the presence of an

only conditions at the present moment under which one would like

Dick Yes, if you use a heterologous type you can sort things out, but if you use a homologous type you can never sort out which paralytic cases might have been due to your vaccine virus, and which had been due to the natural strain, unless you have an adequate marker

Goffe Even so, the use of such a strain might well act only by an interference phenomenon One could argue that one might get exactly the *same* result if material containing interferon were fed, perhaps the right thing to do is a controlled experiment using

sidering the fact that Jennerian vaccination is a dangerous procedure

Ireland.

Kilbourne What Prof Dick wants in the form of the non-spreading virus might theoretically be available simply by giving raw nucleic acid, if this is ever quantitatively possible

Dick It might pick up its host protein and come out the way it started.

Kilbourne This would have to be one with no human potential whatsoever

Goffe Sir Macfarlane, what is the actual evidence for the avirulence of the Malta strain for the years preceding 1943 ?

Dick Why should you do any damage in any immunizing procedure?

Burnet I doubt whether any immunizing procedure is possible without some risk of damage Jennerian vaccination has definitely produced a certain proportion of damage I might say I am strongly in favour of sticking to Salk's!

[illegible]

somebody

[illegible]

these instances adequate evidence of alteration of the genetic con-

[illegible]

of the parasite?

Isaacs I am sure that both you and Dr Kilbourne are in an unassailable position because none of us can produce the 1918 virus for you, and if we could it could not possibly be tested out

Burnet I would stress, with reference to the Malta situation that the cases of *polio in the years preceding* 1942–43 occurred in the

is much greater

Isaacs Could not this be explained on the basis of a different antigenic type?

Burnet I do not think so

a satisfactory one, namely the intracerebral pathogenicity of the

strain of, say, good antigenicity with one that is manifestly avirulent (at least in a laboratory species) then in this condition—where one

transfer virulence and avirulence across to another antigenic site

will emerge as a new dominant form General experience of virus genetics suggests that there can be no such thing as a completely stable virus

Kilbourne I was thinking more specifically in terms of translation [illegible]

described

Smith [illegible]

and other viruses, but I cannot see that he would ever be satisfied, because no matter how long a virus may have remained avirulent under experimental conditions, as soon as it is introduced into the human population it may be unstable and begin to throw off virulent mutants

Dick I have thought about this in 17D quite a bit, although I have not done an experiment to prove it I think it is quite possible that [illegible]

with regard to 17D

Burnet An alternative answer to Prof Smith's question might be that if you gave the whole population the attenuated polio virus at the same time, i e covered everybody, the chance of a mutant [illegible]

probable risk of not using it

Burnet Quite As in all human action!

• • • •

Burnet This has been a very interesting discussion but I think we all knew that no complete picture of virus virulence could emerge [illegible]

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